centration of 519 mg/100 ml. The role of the single injection of RALS in producing such a marked effect on preventing rejection of the xenografts of rat islets is unknown. The theoretical basis for including RALS in the experimental protocol was that the antiserum might destroy passenger leukocytes that were still present in the rat islets at the time of transplantation.

These findings indicate that a marked prolongation of survival of transplants of rat islets and maintenance of normoglycemia in diabetic mice can be achieved by culture of the rat islets in vitro for 7 days at 24°C prior to transplantation and by administration of a single injection of MALS and RALS into the recipients at the time of transplantation. Eloy *et al.* (*11*) reported recently that transplants of 15-day-old chick pancreases into diabetic rats improved the diabetic state of the recipients for a prolonged period of time whereas transplants of 18-day-old chick pancreas did not produce prolonged improvement. The development of methods for the prevention of immune rejection of islet xenografts without the use of continued immunosuppression may be of assistance in the eventual application of islet transplantation to the treatment of diabetes in man, since the source of islet tissue for human transplants may be a critically limiting factor.

PAUL E. LACY
JOSEPH M. DAVIE
EDWARD H. FINKE

Washington University School of Medicine, Department of Pathology and *Department of Microbiology and Immunology, St. Louis, Missouri 63110*

References and Notes

1. K. J. Lafferty, M. A. Cooley, J. Woolnough, K. Z. Walker, *Science* **188**, 259 (1975); K. J. Lafferty, A. Bootes, G. Dart, D. W. Talmage, *Transplantation* **22**, 138 (1976).
2. D. W. Talmage, G. Dart, J. Radovich, K. J. Lafferty, *Science* **191**, 385 (1976).
3. H. W. Sollinger, P. M. Burkholder, W. R. Rasmus, F. H. Bach, *Surgery* **81**, 74 (1977).
4. P. E. Lacy, J. M. Davie, E. H. Finke, *Science* **204**, 312 (1979); *Transplantation* **28**, 415 (1979).
5. C. Weber, A. Zatrigi, R. Weil, R. McIntosh, M. A. Hardy, K. Reemtsma, *Surgery* **79**, 144 (1976).
6. L. G. Frangipane, T. W. Poole, C. F. Barker, W. K. Silvers, *Transplant. Proc.* **9**, 371 (1977).
7. P. E. Lacy and M. Kostianovsky, *Diabetes* **16**, 35 (1967); P. E. Lacy, M. M. Walker, C. J. Fink, *ibid.* **21**, 987 (1972).
8. A. Lindall, M. Steffes, R. Sorenson, *Endocrinology* **85**, 218 (1969).
9. E. H. Finke, P. E. Lacy, J. Ono, *Diabetes* **28**, 612 (1979).
10. C. B. Kemp, M. J. Knight, D. W. Scharp, W. F. Ballinger, P. E. Lacy, *Diabetologia* **9**, 486 (1973).
11. R. Eloy, K. Haffen, M. Kedinger, J. F. Grenier, *J. Clin. Invest.* **64**, 361 (1979).
12. We appreciate the technical assistance of M. L. Chivetta, S. M. Place, and I. M. Rosas. This work was supported in part by PHS grant AM-01226 and by the Kroc Foundation.

10 March 1980

Assignment of the Murine Interferon Sensitivity and Cytoplasmic Superoxide Dismutase Genes to Chromosome 16

Abstract. *Both hybrids of mouse and human microcells and whole cell hybrids generated by the fusion of primary mouse cells and SV40-transformed human fibroblasts were used to establish the syntenic association of the murine cytoplasmic superoxide dismutase and the interferon sensitivity genes on mouse chromosome 16. This assignment adds two new markers to chromosome 16 and provides another example of an evolutionarily conserved linkage. This finding also provides an animal model both for cellular responsiveness to interferon and for Down's syndrome.*

Murine genes have been assigned to linkage groups and specific chromosomes by a combination of breeding experiments and somatic cell genetic studies. Hybrids of Chinese hamster cells with mouse somatic cells have generally been used in mouse mapping studies, since mouse chromosomes are often segregated (*1*). For certain genetic markers, however, the mouse and Chinese hamster gene products cannot be distinguished from each other, making primate × mouse hybrids desirable for murine gene mapping.

Table 1. SOD-1: assignment to chromosome 16. To ascertain the gene assignment, three different statistical procedures were used, χ^2, ϕ, and OR. The χ^2, used as a method of dependence, rather than a test of significance, was calculated in the ordinary way. The ϕ coefficient, which is insensitive to sample size, is the ratio of the observed χ^2 to the perfect χ^2. It is calculated by dividing the square root of the regular χ^2 by the square root of the sample size. When there exists a perfect association between a marker and a chromosome, the value of ϕ is +1. Zero indicates no association; −1 indicates a negative association. The OR statistic was designed for determining gene assignments (*19*). It is an inverse weighted scoring system which is independent of χ^2. It is calculated by assigning each clone a score of 100 and dividing that value evenly among all chromosomes in concordance with the tested marker. The clones are scored, the scores are added for each chromosome, and then divided by the number of clones. The chromosome with the highest number is considered a candidate for assignment of the gene for SOD-1. Generally an OR score of 7 or above is considered evidence for a positive assignment. Statistical analysis was conducted with computer program ASSIGN, developed for the purpose of gene assignment with material generated by somatic cell hybridization (*19*).

Chromosome	SOD-1/chromosome (number of clones)*				ϕ	χ^2	Overall OR
	+/+	+/−	−/+	−/−			
1	3	5	3	12	0.19	0.83	3.95
2	3	5	2	13	0.28	1.79	3.51
3	7	1	9	6	0.28	1.86	5.11
4	6	2	8	7	0.21	1.03	4.24
5	4	4	3	12	0.31	2.22	3.93
6	2	6	2	13	0.15	0.49	3.23
7	2	6	1	14	0.26	1.55	3.49
8	4	4	4	11	0.23	1.25	3.88
9	3	5	5	10	0.04	0.04	2.71
10	3	5	4	11	0.11	0.29	3.01
11	3	5	3	12	0.19	0.83	3.24
12	5	3	11	4	−0.11	0.29	2.92
13	4	4	1	14	0.50	5.76	4.21
14	3	5	1	14	0.39	3.45	3.72
15	5	3	8	7	0.09	0.18	3.44
16	8	0	2†	13	0.83	15.95	7.90
17	6	2	4	11	0.46	4.96	5.55
18	6	2	8	7	0.21	1.03	4.54
19	5	3	10	5	−0.04	0.04	3.63
X	1	7	4	11	−0.16	0.62	2.56

*For chromosomes, + indicates the presence of at least one copy in more than 20 percent of the cells analyzed. The sensitivity of the starch-gel electrophoresis assay for human SOD-1 is relatively low, as determined by dilution assay (*18*). The electrophoretic assay for murine SOD-1 is similarly insensitive. The following isozymes were analyzed in confirmation of chromosome identification: dipeptidase-1 (E.C. 3.4.—) and isocitrate dehydrogenase (E.C. 1.1.1.42) for chromosome 1; enolase (E.C. 4.2.1.2) for chromosome 4; β-glucuronidase (E.C. 3.2.1.31) for chromosome 5; triosephosphate isomerase (E.C. 5.3.1.1) for chromosome 6; lactate dehydrogenase A (E.C. 1.1.1.27) and glucosephosphate isomerase (E.C. 5.3.1.9) for chromosome 7; adenine phosphoribosyltransferase (E.C. 2.4.2.7) for chromosome 8; mannosephosphate isomerase (E.C. 5.3.1.8) and malate dehydrogenase (E.C. 1.1.1.40) for chromosome 9; tripeptidase 1 (E.C. 3.4.—) for chromosome 10; galactokinase (E.C. 2.7.1.6) for chromosome 11; purine nucleoside phosphorylase (E.C. 2.4.2.1) and esterase 10 for chromosome 14; dipeptidase 2 for chromosome 18; glutamate oxaloacetate transaminase (E.C. 2.6.1.1) for chromosome 19; phosphoglycerate kinase (E.C. 2.7.2.3) and glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) for chromosome X. The method of starch-gel electrophoresis of isozymes including cytosol SOD-1 has been described (*4*, *18*). Since our hybrid cells segregate mouse chromosomes rapidly, chromosome spreads and harvesting of cell pellets for isozyme analysis were generally performed within a week of each other. †These two lines (SxO_2-1 and SxA_2-1) have chromosome frequencies of 26 and 21 percent which are the limit (about 20 percent) for detection for SOD by starch-gel electrophoresis.

0036-8075/80/0711-0285$00.50/0 Copyright © 1980 AAAS

Table 2. Interferon receptor (IF-R): assignment to chromosome 16. The chromosome cutoff level here is 20 percent as for Table 1. The low-level expression of interferon sensitivity gene was scored as +.

Chromosome	IF-R/chromosome (number of clones)				ϕ	χ^2	Overall OR
	+/+	+/−	−/+	−/−			
1	2	6	3	7	−0.06	0.06	2.82
2	3	5	2	8	0.19	0.68	3.14
3	6	2	6	4	0.16	0.45	5.11
4	6	2	7	3	0.06	0.06	4.86
5	3	5	2	8	0.19	0.68	3.14
6	2	6	2	8	0.06	0.06	2.79
7	2	6	1	9	0.20	0.72	3.13
8	4	4	2	8	0.32	1.80	4.21
9	3	5	3	7	0.08	0.11	2.79
10	3	5	3	7	0.08	0.11	2.82
11	3	5	2	8	0.19	0.68	3.16
12	6	2	7	3	0.06	0.06	4.85
13	4	4	1	9	0.44	3.54	4.04
14	3	5	1	9	0.33	1.94	3.42
15	5	3	6	4	0.03	0.01	3.72
16	8	0	2*	8	0.80	11.52	9.19
17	6	2	3	7	0.45	3.60	6.10
18	6	2	6	4	0.16	0.45	5.13
19	5	3	9	1	−0.33	1.94	3.97
X	1	7	2	8	−0.10	0.18	2.48

*Clone SxO_2-1 has a chromosome frequency of 26 percent, which is at the limit for detection for the interferon sensitivity gene. The parent clone, SxO_2 also expresses the interferon sensitivity gene weakly even at a chromosome 16 frequency of 58 percent. Therefore, it is likely that SxO_2-1 represents a false negative for the expression of the interferon sensitivity phenotype. However, we have scored it as a discordant clone. Possible explanations for the discordancy of the second clone (SxP_3) are presented in the footnote to Table 3.

Fusions involving mouse and human cells generally produce hybrids that segregate human chromosomes (*2*), although exceptions have been described (*3*). Hybrids segregating mouse chromosomes are generally made by fusing primary mouse cells with virus-transformed human cells. Alternatively, microcell-mediated gene transfer can be used to transfer small numbers of mouse chromosomes into human cells (*4*).

Superoxide dismutase (SOD; E.C. 1.15.1.1) is an enzyme that catalyzes the destruction of the O_2^- free radical formed during various enzymatic reactions or by ionizing radiation (*5*). The cytosols of eukaryotic cells contain a superoxide dismutase (SOD-1) that is made up of two identical subunits, and contains one Cu^{2+} and one Zn^{2+} per subunit. The mitochondrial form of SOD contains four subunits and is a manganese-containing enzyme (*5*). The murine form of the enzyme is indistinguishable from the hamster form by starch-gel electrophoresis, but can readily be distinguished from the human form. In human cells, the gene for the cytoplasmic form of SOD is located on the long arm of chromosome 21 (*6*, *7*). This gene is linked to a genetic element conferring sensitivity to human interferon (*6*, *8*). Recent evidence suggests that the ability of cells to respond to human interferon is determined by cell surface receptors specific for human interferon (*9*). The linkage of these genes might be conserved in mice. Using whole cell hybrids generated by the fusion of primary mouse cells and SV40-transformed human fibroblasts and microcell hybrids retaining only a few mouse chromosomes, we have established the linkage of the murine SOD-1 and interferon sensitivity genes. In addition, we have assigned these genes to mouse chromosome 16.

Sixteen hybrid cell lines were made by polyethylene glycol fusion of spleen cells from C57BL/6J mice with SV40-transformed human CRL 1223 cells. The CRL 1223 cells are deficient in DNA repair activity (xeroderma pigmentosum, complementation group A) and were obtained from the American Type Culture Collection. They were transformed with SV40 (*10*). Two additional hybrids (G_{31} and d6) were made by fusing microcells isolated from mouse embryo fibroblasts with SV40-transformed CRL 1223 cells. Line HEM 3 was derived from a fusion of mouse microcell with human HeLa cells (*4*). Together with four subclones from three independent whole cell hybrid clones, and one subclone from microcell hybrid G_{31}, 24 hybrid lines were analyzed in our study. They cover the full complement of murine chromosomes except Y. All cells were grown in Dulbecco's modification of Eagle medium with 10 percent fetal bovine serum. Hybrids were selected with $10^{-6}M$ ouabain or irradiation with ultraviolet light.

Twenty-three mouse-human hybrid cell lines segregating mouse chromosomes were analyzed for the expression of SOD-1 with regard to chromosome constitution. The SOD-1 isozymes were separated by starch-gel electrophoresis. The assay for SOD was achieved on the basis of its ability to inhibit free radical chain reduction in which O_2^- is an initiator. The staining solution contained MTT tetrazolium and phenazine methosulfate in $0.25M$ sodium phosphate buffer, *p*H 7.5. Karyotype analyses were performed on approximately 30 cells for each clone by a combination of Giemsa banding and Hoechst 33258 centromeric staining (*11*). To confirm the karyotypic results, cell hybrids were also examined for the expression of 18 isozymes that are mapped in the mouse genome to 13 chromosomes (see Table 1).

Table 1 summarizes a statistical analysis of the SOD-1 and karyotypic data for the lines studied. The positive correlation between the presence of an individual chromosome in a clone and that clone's ability to express murine SOD-1 was computed with three different measures of dependence. In all three cases, the expression of mouse SOD-1 was correlated with the presence of chromosome 16. The assignment of SOD-1 to mouse chromosome 16, and specifically to the region of 16B5-16ter, has also been reported (*12*). Our results also showed that retention of chromosome 16 at a frequency of about 20 percent is the threshold for detecting SOD-1 expression by starch-gel electrophoresis.

Since the SOD-1 and interferon sensitivity genes are linked in the human genome, we screened 20 of our hybrids for sensitivity to mouse interferon. Mouse A9 control cells and hybrid cells were exposed to serial dilutions of mouse interferon and challenged with vesicular stomatitis virus (*13*). Hybrid cells were generally less sensitive to mouse interferon than A9 cells, a result similar to

Table 3. Concordant segregation of the expression of mouse SOD-1 and mouse interferon sensitivity gene.

		Interferon receptor	
		+	−
SOD-1	+	7	1*
	−	1†	9

*Clone SxP_3 retains chromosome 16 at a frequency of 59 percent and expresses murine SOD-1, but does not respond to mouse interferon. A higher dose of interferon may be required to trigger the antiviral state in this line. Alternatively, this discrepancy could be due to chromosome breakage or chromosomal translocation. †This line, $SxA_2 \times 1$, weakly expresses interferon sensitivity, and has a retention frequency of 21 percent for chromosome 16. We believe this clone is a false negative for SOD-1 expression.

that observed for human-mouse hybrid cell sensitivity to human interferon (*14*). Interferon was assayed within 1 week of karyotype and isozyme analysis. As before, sensitivity to mouse interferon could be assigned to chromosome 16, and it segregated concordantly with mouse SOD-1 (Tables 2 and 3). Thus, when SOD-1 negative subclones of previously SOD-1 positive hybrids were examined, they had invariably lost both sensitivity to murine interferon and mouse chromosome 16.

Numerous reports of the preservation of synteny relationships in sets of homologous genes in a number of mammalian species suggest that evolution may have favored the retention of some ancestral gene linkages. The linkage of the mouse genes for sensitivity to mouse interferon and for cytoplasmic SOD represents the same linkage described for the corresponding human genes. Sensitivity to human interferon and human SOD-1 have both been mapped to a small region of human chromosome 21 (*15*). SOD-1 has been assigned to chromosome segment 21q22 (*7*), while a gene governing response to human fibroblast, leukocyte, and immune interferons has been mapped to the distal portions of the long arm of this chromosome (q21-qter) (*16*). The region of chromosome 21 containing these two genes represents a small portion of the human genome (about 0.5 percent) and the linkage of these genes might be expected to be conserved in other mammalian species. Data on one microcell hybrid we examined (d6) suggest that the genes for SOD-1 and mouse interferon sensitivity are also closely linked. Even though no murine genetic material can be cytologically detected in this line, isozyme analysis and mouse interferon sensitivity assays indicate that the two genes are present and expressed.

The mapping of SOD-1 and a gene for interferon sensitivity to mouse chromosome 16 adds two new markers to this chromosome and provides another example of an evolutionarily conserved linkage. Comparative mapping data such as these can provide valuable information about chromosome evolution and organization. The assignment of the mouse interferon gene can also be expected to contribute in a significant way to the advance of experimental studies on the mechanism of interferon action. Moreover, it is known that an extra copy of human chromosome 21 (trisomy 21) is the genetic basis for Down's syndrome. The specific genes involved in this condition have not been specified, but it is known that the crucial genetic information resides in band 21q22 (*17*). The demonstration that murine SOD-1 and interferon sensitivity are linked and that their human homologs reside in a region which is correlated with Down's syndrome expression, suggests that partial trisomy in mouse 16 could possibly serve as an animal model for this important human genetic disease.

PIN-FANG LIN
DORIS L. SLATE
FRANCES C. LAWYER
FRANK H. RUDDLE

Department of Biology,
Yale University,
New Haven, Connecticut 06520

References and Notes

1. C. A. Kozak and F. H. Ruddle, *In Vitro* **12**, 720 (1976); L. Leinwand, E. Nichols, F. H. Ruddle, *Biochem. Genet.* **16**, 659 (1978).
2. F. H. Ruddle and R. P. Creagan, *Annu. Rev. Genet.* **9**, 407 (1975).
3. C. M. Croce, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3248 (1976); E. M. Frankfort, E. A. Havell, C. M. Croce, J. Vilcek, *Virology* **89**, 45 (1978).
4. C. A. Kozak, R. E. K. Fournier, L. A. Leinwand, F. H. Ruddle, *Biochem. Genet.* **17**, 23 (1979).
5. I. Fridovich, *Annu. Rev. Biochem.* **44**, 147 (1975).
6. Y. H. Tan, J. A. Tischfield, F. H. Ruddle, *J. Exp. Med.* **137**, 317 (1973).
7. T. Philip, J. Fraisse, P. M. Senet, B. Lauras, J. M. Robert, F. Freycon, *Cytogenet. Cell Genet.* **22**, 521 (1978).
8. C. Chany, M. Vignal, P. Couillin, N. V. Cong, J. Boue, A. Boue, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3129 (1975).
9. M. Revel, D. Bash, F. H. Ruddle, *Nature (London)* **260**, 139 (1976); C. Chany, *Biomed. Express (Paris)* **24**, 148 (1976).
10. J. A. McDougall, Hutchinson Cancer Center, Seattle, Wash.
11. C. A. Kozak, J. B. Lawrence, F. H. Ruddle, *Exp. Cell Res.* **105**, 109 (1979).
12. U. Francke and R. T. Taggart, *Proc. Natl. Acad. Sci. U.S.A* **76**, 5230 (1979).
13. D. L. Slate and F. H. Ruddle, *Cell* **16**, 171 (1979).
14. D. L. Slate, L. Shulman, J. Lawrence, M. Revel, F. H. Ruddle, *J. Virol.* **25**, 319 (1978).
15. Y. H. Tan and A. E. Greene, *J. Gen. Virol.* **32**, 153 (1976).
16. L. B. Epstein and C. J. Epstein, *J. Infect. Dis.* **133**, A56 (1976).
17. A. Hagemeijer and E. M. E. Smit, *Hum. Genet.* **38**, 15 (1977).
18. E. A. Nichols and F. H. Ruddle, *Biochem. Genet.* **17**, 127 (1979).
19. E. A. Nichols and F. H. Ruddle, *J. Histochem. Cytochem.* **21**, 1066 (1973).
20. M. Cowmeadow and F. H. Ruddle, *Cytogenet. Cell Genet.* **22**, 694 (1978).
21. We thank Dr. C. Kozak for her HEM hybrid line; Dr. P. Lengyel for the mouse interferon; Drs. P. D'Eustachio, D. Pravtcheva, and L. Klobutcher for helpful discussion; U. Shankar for performing statistical analysis; E. Nichols and V. Salerno for performing isozyme assays; and D. Bonito for technical assistance. Supported by an American Cancer Society grant and NIH grant GM 09966 (F.H.R.).

22 October 1979; revised 26 December 1979

Mammoth Albumin

Abstract. *Serum albumin was detected immunologically in muscle from a mammoth that died about 40,000 years ago. Rabbits injected with ground mammoth muscle produced antibodies that react strongly with elephant albumin, weakly with sea cow albumin, and still more weakly or not at all with other mammalian albumins. Since elephant albumin elicited antibodies with the same specificity, some of the surviving mammoth albumin molecules evidently have antigenic sites identical to those on native elephant albumin. Much of the mammoth albumin has, however, undergone postmortem change. The small amount of soluble albumin extractable from mammoth muscle is heterogeneous in size, charge, and antigenic properties.*

Many fossils contain remnants of proteins whose amino acid sequences presumably carry information about the structure of the genes of extinct creatures. However, previous attempts to extract genetic information from fossil proteins have met with little success (*1*). With a new immunological approach, we have found a globular protein in mammoth remains and estimated its genetic relatedness to the proteins of living species (*2*). The investigation of proteins from such well-preserved fossils as mammoths (*3*) may serve as a stepping-stone to the genetic study of less well-preserved fossils.

Not surprisingly, the globular protein we detected in mammoth tissue is albumin. Albumin is an excellent candidate for survival and immunological detection in vertebrate fossils because of its high concentration in all tissues (*4, 5*), resistance to denaturation (*4-6*), and immunogenicity (*7*). Further, albumin has been used for making genetic comparisons of about 2000 pairs of living species (*7*), and this large body of comparative information provides a framework for analyzing data obtained from the albumins of extinct species.

Frozen muscle from the right thigh of a 40,000-year-old mammoth (*Mammuthus primigenius*) (*8*) was ground in buffer, and the resulting suspension was injected into rabbits (*9*). The antiserums produced after 12 weeks of immunization were tested for reactivity with the soluble proteins in serum and an extract of leg muscle of an Indian elephant. In the immunodiffusion test (*10*), each antiserum produced a single precipitin line with either serum or the muscle extract. The fusion of the two lines indicated that the rabbits had made antibodies chiefly toward a single mammoth protein. This protein was identified as albumin in two ways (Fig. 1). First, each antiserum gave a precipitin line with pure elephant albu-

0036-8075/80/0711-0287$00.50/0 Copyright © 1980 AAAS