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.

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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-

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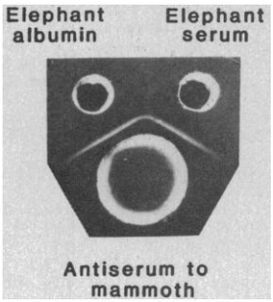

Fig. 1. Immunodiffusion test showing that an antiserum to mammoth muscle contains antibodies to albumin. The antiserum to mammoth muscle is shown to react with pure Indian elephant albumin as well as with Indian elephant serum. The fusion of the two precipitin lines indicates that the principal component of elephant serum with which antiserum to mammoth muscle reacts is albumin. For this test the antiserum was concentrated fivefold by vacuum dialysis and a large antiserum well (5.5 mm in diameter instead of 2 mm) was used because of the low concentration of antibodies. The purified, elephant albumin was at a concentration of 610 μg/ml, and the serum was diluted 100-fold.

min (*11*). Second, the albumin line fused with the line produced between antiserum and either serum or muscle extract. The reaction of mammoth antiserum with elephant albumin was confirmed by microcomplement fixation (*12*), radioimmunoassay (*13*), and immunoelectrophoresis.

The specificity of the strongest antiserum to mammoth muscle was then compared with that of antiserums to albumin purified from Indian and African elephants (*14*). According to immunodiffusion and microcomplement fixation tests, the specificities are nearly identical. All three kinds of antiserums reacted strongly with albumin from the two living species of elephant, weakly with sea cow albumin (*15*), and still more weakly or not at all with the albumins of species belonging to other orders of mammals. The results of a quantitative microcomplement fixation test with antiserum to mammoth albumin appear in Fig. 2: Indian and African elephant albumins each fix 87 percent of the available complement under conditions where sea cow fixes virtually none. Antiserums to either elephant albumin also gave virtually the same results. Furthermore, when higher concentrations of any of the three antiserums were used, a reaction with sea cow albumin was obtained; and in every case the immunological distance (*2, 12*) from elephant to sea cow albumin was about 140.

The near identity of the specificities of the three antiserums in these quantitative tests implies that mammoth albumin is as closely related to Indian and African elephant albumins as are the two elephant albumins to each other. This is consistent with anatomical evidence concerning the kinship of mammoths to elephants (*16*).

We next conducted direct tests with the small amount of soluble albumin extractable from mammoth muscle (*17*). Soluble albumin was detected by both radioimmunoassay (*13*) and immunodiffusion (*10*). Its concentration in mammoth muscle extracts was estimated to be about 50 times lower than expected for an extract made from a comparable amount of elephant muscle.

Much of the soluble mammoth albumin has undergone postmortem change, as is evident from its behavior during filtration through a column of Sephadex G-100, which separates proteins according to size. About 80 percent of the albumin was aggregated according to radioimmunoassay of fractions emerging from the column. Only about 20 percent of the albumin appeared to be similar in size to native monomeric albumin. Additional tests suggest that soluble mammoth albumin is heterogeneous not only in size but also in charge and antigenic specificity.

To examine the antigenic relation of this heterogeneous mixture of soluble mammoth albumins to albumin from living species, we used antiserums made against the albumins of numerous mammalian species. Results obtained with the immunodiffusion test indicate a close relation between mammoth and elephant albumins, a distant relation with sea cow albumin, and a more distant relation with other albumins. Though these results parallel those obtained by comparison of the specificity of antiserums to mammoth and other albumins, there is a significant difference between the two sets of data. Whereas the antiserums to mammoth and elephant albumin have the same specificity, soluble mammoth albumin differs antigenically from elephant albumin. With antiserums to elephant albumin (Fig. 3), the precipitin line formed by elephant albumin fails to fuse fully with the line given by mammoth albumin. This reaction of partial identity implies that soluble mammoth albumin does not bind all of those antibodies that can precipitate elephant albumin. The difference between mammoth and elephant albumin is unexpectedly large (*18*), which we tentatively attribute to postmortem modification of most of the soluble mammoth albumin molecules.

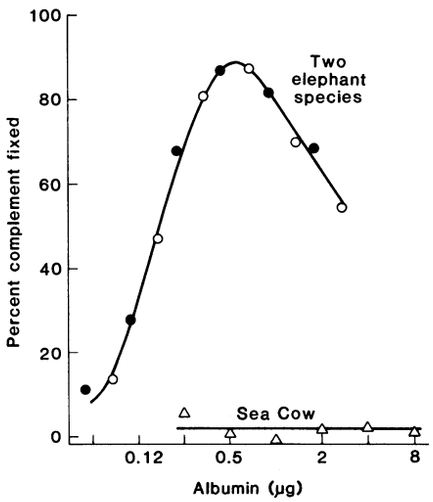

Fig. 2. Specificity of an antiserum to mammoth albumin, according to the microcomplement fixation test (*12*). At the concentration used, this antiserum reacts far better with albumin of the Indian elephant (●) and African elephant (○) than with that of the sea cow (△), *Trichechus manatus*. The same antiserum concentration was used for all reactions: each tube received 1 ml of a 1:50 dilution of antiserum, the final volume of the reaction mixture being 6 ml. The antigens were pure albumin in the case of the Indian elephant and serum in the case of the other two species. The albumin content of the serum was assumed to be 30 mg/ml.

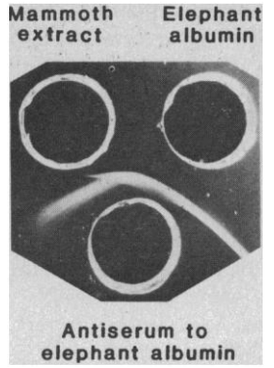

Fig. 3. Immunodiffusion evidence for soluble mammoth albumin and the demonstration of a difference between mammoth and elephant albumins. An antiserum to Indian elephant albumin is shown to react with the mammoth extract and elephant albumin. The elephant line forms a spur over the mammoth line. The antigens were concentrated mammoth extract (*17*) and pure Indian elephant albumin (5 mg/ml), and the antiserum was concentrated fivefold by vacuum dialysis. When an analogous test was done with antiserum to African elephant albumin (results not shown), a spur was again observed in the comparison of mammoth extract and elephant albumin.

Our study has shown two contrasting ways of using antibodies to examine proteins of extinct creatures. One is the conventional, direct approach of reacting antibodies with fossil organic material (*13, 19*). Postmortem changes can make interpretation of these reactions difficult. The second approach, introduced in this report, may be termed indirect because no antigenic tests are done with the fossil material. Instead, this material is injected into rabbits and the resulting anti-

serums are tested only with the proteins of modern creatures. By quantitatively comparing the specificities of these antiserums with those of antiserums to the purified proteins of living species, one can in theory determine the genealogical relations of extinct creatures to living ones. An attractive feature of the indirect method is that it focuses attention on those fossil molecules which have been least modified.

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17. The soluble extract was made by grinding 3 g of muscle in 50 ml of isotris buffer, *p*H 7.45 (*12*), and concentrating the supernatant 100-fold by vacuum dialysis and lyophilization.
18. Morphologists think that the mammoth is as closely related to the two living elephant species as they are to each other (*16*). The identity of the two elephant albumins in both microcomplement fixation and immunodiffusion tests implies a 0 to 1 percent sequence difference (*2*). But the spur seen with soluble mammoth albumin in Fig. 3 implies a 6 percent sequence difference between mammoth and elephant albumins (*2*).
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Genes for Growth Hormone, Chorionic Somatomammotropin, and Growth Hormone–Like Gene on Chromosome 17 in Humans

Abstract. *The human genes for growth hormone* (GH), *chorionic somatomammotropin* (CSH), *and a third growth hormone–like gene* (GHL) *have been located on chromosome 17 in humans. DNA fragments of 2.6, 2.8, and 9.5 kilobase pairs containing* GH, CSH, *and* GHL, *respectively, were identified in human genomic DNA, and a 7.5-kilobase DNA fragment related to growth hormone DNA sequences was found in mouse cells. In somatic hybrids of human and mouse cells containing reduced numbers of human chromosomes, but a normal complement of mouse chromosomes, the mouse 7.5-kilobase DNA fragment was always present, whereas the 2.6-, 2.8-, and 9.5-kilobase human fragments were present only when human chromosome 17 was also present.*

The human polypeptide hormones growth hormone and chorionic somatomammotropin (also called placental lactogen) (*1*) are produced in the pituitary and placenta, respectively, yet they are closely related. They are composed of 191 amino acid residues and show about 85 percent amino acid-sequence homology (*2*). Their messenger RNA's (mRNA's) have more than 90 percent homology (*3, 4*). The human genes coding for growth hormone and chorionic somatomammotropin have been cloned, and DNA sequence analysis indicates that the genes have similar intervening sequences (*5*).

A second pituitary hormone, prolactin, shares to a lesser degree a structural homology with growth hormone and chorionic somatomammotropin (*6*). All three polypeptide hormones are believed to originate from a common ancestral gene by gene duplication (*4, 7, 8*) and thus may be closely linked on the chromosome.

The growth hormone gene (*GH*), the chorionic somatomammotropin gene (*CSH*), and a third growth hormone–like gene (*GHL*) are located on human chromosome 17. We used somatic cell hybrids of human and mouse cells to determine these locations. Cell hybrids were constructed and maintained by methods previously described (*9–11*); they contain a normal complement of mouse chromosomes, but the human complement contains reduced numbers and different combinations of human chromosomes. Cell hybrids containing 3 to 22 different human chromosomes were examined (Table 1).

Large-molecular-weight DNA was isolated from human T cell lymphoblasts, from the RAG mouse cell line, and from human-mouse cell hybrids by the method previously described (*11*). DNA (10 to 20 μg) was then digested to completion by the restriction endonuclease Eco RI. Digested fragments were separated by electrophoresis through a 1 percent agarose gel and transferred to nitrocellulose filters by the method of Southern (*12*).

The construction of the recombinant plasmid chGH800/pBR322, which contains DNA complementary to nearly full-length human growth hormone mRNA, has been previously discussed (*4*). The 800-nucleotide growth hormone complementary DNA (cDNA) insert was isolated from pBR322 plasmid DNA by Hind III digestion and preparative gel electrophoresis (*4*). A ^{32}P-labeled probe was made by labeling the insert by the method of random priming with calf thymus DNA primers (*13*). Specific activities greater than 10^8 cpm/μg were

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