The serological analysis and the restriction enzyme pattern of the tick isolates leave little doubt that the viruses were IBR virus. To our knowledge, this is the only reported example of a mammal-infecting herpesvirus isolated from an arthropod host. It is not known whether the presence of the virus in the ticks is a result of biological replication or mechanical transmission. The source of the virus that infected the ticks is also in question. We have found antibodies to IBR virus in both deer and cattle in the area of the Sierra Nevada (*9*) where the ticks were collected. Since the viremic stage of disease in an animal is relatively short, there must be very few animals in an area at a particular time capable of transmitting virus to ticks; consequently, it is puzzling that virus was isolated from three separate collections of ticks obtained over a 3-year period. The presence of IBR virus in each of these collections would be even more remarkable if the ticks were mechanically infected, since one would expect the virus to be present for only brief intervals. Hence it is important to determine (i) whether IBR virus can replicate in ticks, (ii) how long the virus remains infectious in ticks, (iii) whether ticks can be infected with IBR virus from feeding on viremic cattle and can then transmit the disease to normal cattle, (iv) whether IBR virus can be observed in the tick with the electron microscope, and (v) whether the virus can be mechanically transmitted without replication of the virus.

ROBERT E. L. TAYLOR
Agriculture Experiment Station, University of Nevada School of Veterinary Medicine, Reno 89502
BRUCE S. SEAL
STEPHEN ST. JEOR
Department of Microbiology, University of Nevada School of Medicine, Reno 89557

References and Notes

1. S. B. Mohanty, *Adv. Vet. Sci. Comp. Med.* **22**, 83 (1978).
2. R. F. Kahrs, *J. Am. Vet. Med. Assoc.* **171**, 1055 (1977).
3. A. Wiseman, P. M. Msolla, S. E. Selman, E. M. Allan, H. M. Pirie, *Vet. Rec.* **107**, 436 (1980).
4. T. L. Chow and R. W. Davis, *Am. J. Vet. Res.* **25**, 518 (1964).
5. R. Garcia, *Ann. Entomol. Soc. Am.* **55**, 605 (1962).
6. E. D. Kieff, S. L. Bachenheimer, B. Roizman, *J. Virol.* **8**, 125 (1971).
7. J. Skare, W. P. Summers, W. C. Summers, *ibid.* **15**, 726 (1975).
8. E. S. Huang *et al.*, *ibid.* **12**, 1473 (1973).
9. R. L. Taylor, unpublished data.
10. We thank C. Hall, J. Martinez, R. Ota, M. Hanks, and K. Spencer for their assistance in preparing the manuscript. These studies were supported by RO1-CA28089 from the National Institutes of Health, a grant from the Reno Cancer Society, and the Research Advisory Board of the University of Nevada. This is a contribution of the Nevada Agricultural Experiment Station, Journal Series No. 543.

11 February 1981

Human Immunoglobulin Heavy Chain Genes Map to a Region of Translocations in Malignant B Lymphocytes

Abstract. *A human immunoglobulin heavy chain (γ4) gene is mapped by chromosome hybridization in situ. This gene is located at band 14q32, a site commonly involved in a chromosomal translocation characteristic of malignant B cells.*

Two types of genetic rearrangements are associated with certain malignant B (bone marrow derived) lymphocytes. One involves the rearrangements necessary to form active immunoglobulin genes (*1*). The other consists of less well understood chromosomal translocations observed in certain human B cell lymphomas and leukemias, translocations that join a part of chromosome 8 to one of the chromosomes (namely, 2, 14, or 22) that bear immunoglobulin genes (*2*). The association of these nonrandom translocations with the chromosomes that carry immunoglobulin determinants suggested that these recombinational events might be related (*3*). Klein (*4*) and Rowley (*2*) have postulated how these translocations might also induce or maintain the malignant state.

Initial experiments (*5*) with human hybrid cell lines had resulted in the assignment of the human genes coding for immunoglobulin heavy, κ, and λ chains to chromosomes 14, 2, and 22, respectively. Chromosomal hybridization techniques in situ (*6–8*) now allow us to map these genes to their chromosomal bands with greater precision. We have begun by mapping the human heavy chain constant region gene, γ4. We find that it is located on chromosome 14, band q32 (14q32), exactly the band to which a characteristic B cell lymphoma and leukemia translocation occurs.

The probe for these mapping studies consisted of a 6.6-kilobase (kb) cloned human DNA fragment that, by sequence analysis, has been shown to include the human γ4 constant region (Igγ4) gene and its associated flanking sequences. This fragment has been physically linked to genes corresponding to other γ subclasses and bears considerable homology to them (*9*). The fragment contains no reiterated sequences. It was subcloned in the plasmid pBR322 (Fig. 1), labeled with ^3H, and used as a probe of human mitotic chromosome spreads in an in situ hybridization reaction (*10*).

The appearance of a quinacrine-banded chromosome spread visualized with incident light fluorescence is shown in Fig. 2A. Under added visible light and when the plane of focus is raised to the level of the emulsion (Fig. 2B), a silver grain (arrow) is seen at the distal segment of the q (long) arm of one of the chromosomes 14 in this cell. The chromosome banding pattern stays clear for 1 to 2 minutes before fading. A burning out of the background fluorescence creates a circular halo around the chromosome spreads, thus making it easy to distinguish previously analyzed from unanalyzed spreads. Since the stain is fluorescent, the silver grains are clearly visualized against the green-white chromosomal background.

A histogram presenting data compiled from an analysis of 50 chromosomal spreads is shown in Fig. 3. The data represent the analysis of two observers working independently, one of whom was unaware of the identity of the hybridizing probe. The recordings by the two observers were essentially identical, and the experiment has been repeated with peripheral blood from three different sources. Between one and five grains were associated with chromosomes in each chromosome spread. The grain background was either in the same range or, in most of the spreads, lower than the number associated with chromosomes. Of the 50 cells represented in the histogram more than 60 percent contained at least one chromosome 14 with a silver grain at the distal end of the q arm. Approximately 30 percent of all the grains observed were located at 14q32.

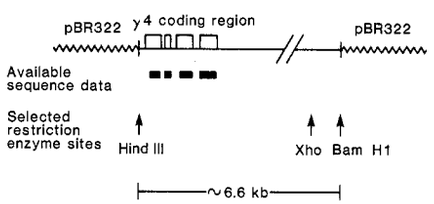

Fig. 1. Cloned human γ4 DNA fragment used as in situ probe. A human γ4 DNA segment was isolated from a partial Mbo I library (*21*) of human placental DNA fragments, packaged (*22*) in the λCH28 vector (*23*), and identified by in situ hybridization (*24*) with a previously cloned human γ4 DNA fragment that was initially identified through cross-hybridization to a cloned mouse γ chain cDNA probe (*25*). An approximate 6.6-kb Bam HI–Hind III fragment was subcloned into the plasmid pBR322. DNA sequence determination (*26*) in the first, second, and third domains and hinge region (filled boxes, data not shown) identified this subclone as γ4 genomic fragment when compared to known amino acid sequences of heavy chain constant regions (*27*).

0036-8075/82/0416-0301$01.00/0 Copyright © 1982 AAAS

Poisson distribution analysis shows that the grain distribution over 14q32 is very significant ($P << .01$).

Since this same probe shows strong cross-hybridization with other γ subclasses on other genomic clones that we have isolated (*9*), it is likely that the target for hybridization is not a single gene but rather the entire γ heavy chain constant region locus. Our in situ hybridization with a cloned and sequenced human μ heavy chain constant region gene (*11*) is yielding data essentially identical to that presented here. Therefore, by analogy to physical linkage studies in the mouse (*12*), other heavy chain constant region genes should be located at band 14q32. If one extrapolates from the mouse data where the heavy chain constant region locus extends over a 150- to 200-kb distance, the human constant region should occupy roughly 2 percent of the genetic material within this band (there are, on average, about 10^4 kb per band for each of the 400 bands that can be recognized on chromosomes at this stage of metaphase). We have been informed of heavy chain allotype mapping studies with cells from a patient with a ring chromosome 14 which corroborate our localization (*13*).

Specific chromosomal aberrations are associated with distinct acquired diseases (*3, 14*). Extensive correlations have been observed in analyses of various human leukemias and lymphomas (*15*), the best known being the occurrence of the "Philadelphia" chromosome in chronic myelogenous leukemia. Evidence has been accumulating that most, if not all, Burkitt's lymphomas and some B-cell leukemias are associated with a translocation from chromosome 8 onto chromosome 14, 2, or 22 with the translocation from 8 to 14 being most common (*2, 16*). Whether this translocation plays a role in instigating the disease process or occurs subsequent to the malignant transformation is not clear. The mechanisms by which such a translocation could induce the malignant state, for example, by moving an oncogene into a transcriptionally active locus, have been considered by others (*2, 4, 17*). Of relevance to our study is the fact that the site of translocation on chromosome 14 is band q32, the same band to which our Igγ4 probe maps. The site of translocation onto chromosome 2 is the band where the κ light chain genes map (*2, 5, 6*).

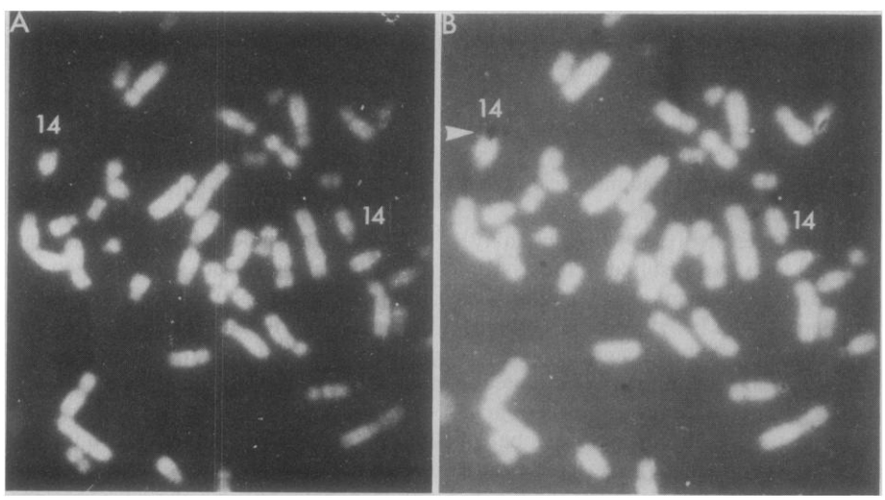

Fig. 2. Photographs of a metaphase chromosome spread in situ hybridized with γ4 probe. (A) Chromosomes of the cell illuminated by ultraviolet light. (B) Under added visible light, a silver grain appears at the distal tip of one of the two chromosomes 14 of the cell. The method for chromosome spreading and staining and the in situ hybridization is described in (*10*).

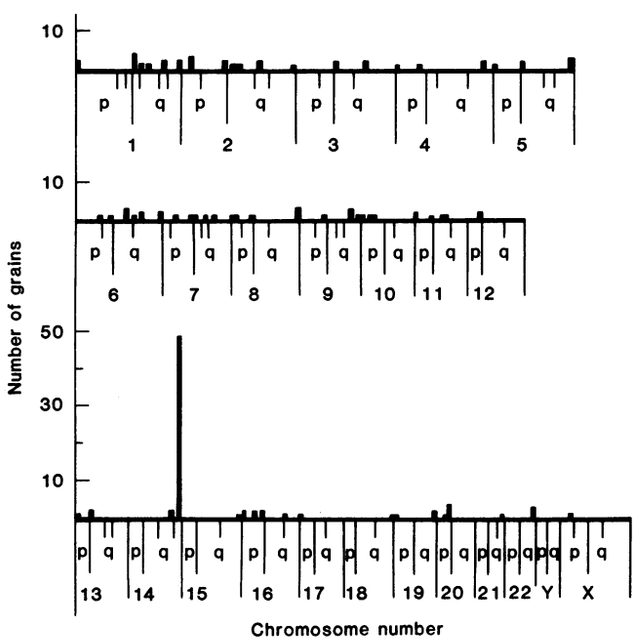

Fig. 3. Distribution of silver grains over metaphase chromosomes, obtained from an analysis of 50 cells. A significant grain accumulation is noted over the distal tip of the q arm of chromosome 14, band 14q32.

This coincidence could be (i) fortuitous, (ii) related to the transcriptional activity of this area in B lymphocytes, or (iii) specifically related to the recombinational properties of the genes affected by the translocation. The immune system requires the somatic rearrangement of DNA during its normal activation (*18*). These genes undergo at least two types of somatic rearrangement, each of which is likely to be mediated by separate genetic signals and different recombinational enzymes. One recombinational reaction involves the joining of the V (variable) and J (joining) region segments [or V, D (diversity), and J region segments] to form a complete variable region gene (*1*). The second involves the switching of a complete variable region gene to different genes of the heavy chain constant region (*1, 19*). This second reaction, called the heavy chain class switch, is thought to be mediated by special nucleotide sequences (switch signals) encoded near each heavy chain constant region gene. Each of these recombinational mechanisms expands genetic diversity in such a powerful way that it is reasonable to imagine that they are used to generate diversity in other genetic systems. Indeed, we have already shown that many switch-like signals map outside the heavy chain locus (*20*). Conceivably a V-J or switch-like recombinational mechanism could promote the translocations that consistently involve the chromosomes that bear immunoglobulin genes and chromosome 8 in malignant B cells.

The mapping of the immunoglobulin genes to the precise bands involved in these translocations opens the way for further experimental tests.

ILAN R. KIRSCH
Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland 20205

CYNTHIA C. MORTON
Department of Human Genetics, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

KENNETH NAKAHARA
Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland 20205

PHILIP LEDER
Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, and *Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115*

References and Notes

1. E. Max, J. Seidman, P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3450 (1979); H. Sakano, K. Huppi, G. Heinich, S. Tonegawa, *Nature (London)* **280**, 288 (1979); J. Seidman, E. Max, P. Leder, *ibid.*, p. 370; P. Early, H. Huang, M. Davis, K. Calame, L. Hood, *Cell* **19**, 981 (1980); H. Sakano, R. Maki, Y. Kurosawa, W. Roeder, S. Tonegawa, *Nature (London)* **286**, 676 (1980).
2. For a review of information pertaining to these translocations see J. Rowley, *Science*, in press.
3. S. Ohno, M. Babonits, F. Wiener, J. Spira, G. Klein, M. Potter, *Cell* **19**, 1001 (1979).
4. G. Klein, *Nature (London)* **294**, 313 (1981).
5. C. Croce *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3416 (1979); J. Erikson, J. Martinis, C. Croce, *Nature (London)* **294**, 173 (1981); O. McBride, P. Hieter, G. Hollis, D. Swan, M. Otey, P. Leder, *J. Exp. Med.*, in press; S. Malcolm, P. Barton, D. Bentley, M. Ferguson-Smith, C. Murphy, T. Rabbitts, *Human Gene Mapping Conference VI, 1981* (National Foundation–March of Dimes, New York, in press); C. Croce *et al.*, *Ann. Hum. Genet.*, in press.
6. J. Gall and M. L. Pardue, *Proc. Natl. Acad. Sci. U.S.A.* **63**, 378 (1969); D. Gerhard, E. Kawasaki, F. Bancroft, P. Szabo, *ibid.* **78**, 3755 (1981); S. Malcolm, P. Barton, C. Murphy, M. Ferguson-Smith, *Ann. Hum. Genet.* **45**, 135 (1981).
7. M. Harper and G. Saunders, *Chromosoma* **83**, 431 (1981).
8. S. Lawrie and J. Godsen, *Hum. Genet.* **53**, 371 (1980).
9. I. R. Kirsch, unpublished data.
10. The γ4 subclone including pBR322 vector was ^{3}H-labeled by nick-translation with ^{3}H-labeled deoxyadenosine triphosphate (dATP) (46.2 Ci/mmole), ^{3}H-labeled deoxycytidine triphosphate, (dCTP) (54.1 Ci/mmole), and ^{3}H-labeled deoxythymidine triphosphate (dTTP) (94 Ci/mmole) (New England Nuclear) in the presence of excess deoxyguanosine triphosphate (dGTP). The probe was separated from unreacted ^{3}H-labeled deoxynucleotides by elution through a 12-ml Sephadex G-50 column (Pharmacia). The specific activity of the probe was 1×10^7 cpm/μg. Human peripheral blood (10 drops per flask in 10 ml of RPMI 1640 media, 20 percent fetal calf serum, glutamine, penicillin, streptomycin, and neomycin) was incubated in the presence of 0.2 ml of phytohemagglutinin (Difco) for 62 to 76 hours at 37°C (5 percent CO_2). Two hours before harvest, Colcemid (0.08 μg/ml) was added. The cells were harvested, treated with hypotonic 0.075*M* KCl, washed, fixed with methanol-acetic acid (3:1), and dropped from a height of 18 to 36 inches onto clean cold wet slides. The slides were dried in air and used for in situ hybridization within 2 weeks. The hybridization was like that described in (*7*) up to the chromosome banding. The slides were first treated with boiled ribonuclease (Sigma; 100 μg/ml) in 2 × SSC (0.3*M* NaCl, 0.03*M* disodium citrate, *p*H 7.0) and incubated at 37°C for 1 hour, rinsed four times in 2 × SSC, and dehydrated in an ethanol series. Chromosomal DNA was denatured by immersion of the slides in 70 percent formamide, 2 × SSC for 2 minutes at 70°C, and subsequently dehydrated in ethanol. The ^{3}H-labeled γ4-pBR322 probe (0.2 μg/ml) was denatured by heat (70°C) for 5 minutes [in 50 percent formamide, 2 × SSCP (0.3 ml NaCl, 0.03*M* disodium citrate, 0.04*M* $NaPO_4$, *p*H 6.0), 10 percent dextran sulfate (Pharmacia), and sonicated DNA from *Escherichia coli* (50 μg/ml)] and quickly cooled. The final *p*H was 7.0 to 7.4. Portions of the probe (25 to 30 μl) were applied to each slide. Cover slips (24 by 50 mm) were placed on the slides and the slides were incubated (12 to 18 hours at 37°C) in a sealed box with a humid environment, then rinsed three times in 50 percent formamide 2 × SSC, *p*H 7.0 at 39° to 40°C, washed five times in 2 × SSC at 39° to 40°C, and dehydrated in ethanol. Some of the slides were first stained in quinacrine dihydrochloride (Sigma Q250) (*8*); all slides were dried in air, covered with Kodak NTB2 nuclear track emulsion, and incubated in light-tight containers with desiccant at 4°C for 10 to 21 days. The slides were developed in Kodak D19 developer (1:1 with H_2O) at 15°C for 4 minutes, rinsed in twice-distilled water, treated with Kodak fixer for 5 minutes, soaked in twice-distilled water for 5 minutes, and air-dried. The dried slides were stained with quinacrine mustard (Sigma Q-2000) at a concentration of 0.005 percent in MacIlvaine's buffer (0.1*M* citric acid, 0.2*M* Na_2HPO_4, *p*H 5.4) for 20 minutes, rinsed ten times twice in MacIlvaine's buffer, and soaked an additional 10 minutes in MacIlvaine's buffer in a light-tight container. The slides were analyzed on Leitz Ortholux II microscopes equipped with incident light fluorescence capacity and 63× oil immersion fluorescent lenses with the use of the broad range blue fluorescence or with K 510 barrier filters. Grain distribution was recorded with reference to a Yunis karyogram [J. Yunis, *Hum. Pathol.* **12**, 494 (1981)] of human G- or Q-banded chromosomes at the 400-band stage.
11. J. Ravetch, U. Siebenlist, S. Korsmeyer, T. Waldmann, P. Leder, *Cell* **27**, 583 (1981).
12. A. Shimizu, N. Takahashi, Y. Yamawaki-Kataoka, T. Honjo, *Nature (London)* **289**, 149 (1981); Y. Nishida *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1581 (1981).
13. D. Cox, personal communication.
14. J. Rowley, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5729 (1977); A. Bernheim, R. Berger, G. Lenoir, *Cancer Genet. Cytogenet.* **3**, 307 (1981).
15. J. Rowley, *Annu. Rev. Genet.* **14**, 17 (1980); in *Genes, Chromosomes and Neoplasia*, F. Arrighi, P. Rao, E. Stubblefield, Eds. (Raven, New York, 1981).
16. L. Zech, U. Haglund, K. Nilsson, G. Klein, *Int. J. Cancer* **17**, 47 (1976); B. Kaiser-McCaw, A. Epstein, H. Kaplan, F. Hecht, *ibid.* **19**, 482 (1977); E. Douglass, I. Magrath, E. Lee, J. Whang-Peng, *Blood* **55**, 148 (1980).
17. W. Hayward, B. Neel, S. Astrin, *Nature (London)* **290**, 475 (1981).
18. D. Levitt and M. Cooper, *Cell* **19**, 617 (1980); P. Hieter, S. Korsmeyer, T. Waldmann, P. Leder, *Nature (London)* **290**, 368 (1981); E. Siden, P. Alt, L. Shunefeld, V. Sato, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1823 (1981); S. Korsmeyer, P. Hieter, J. Ravetch, D. Poplack, T. Waldmann, P. Leder, *ibid.*, p. 7096.
19. M. Davis *et al.*, *Nature (London)* **283**, 733 (1980); T. Kataoka, T. Kawakami, N. Takahasi, T. Honjo, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 919 (1980); R. Maki, A. Trannecker, H. Sakano, W. Roeder, S. Tonegawa, *ibid.*, p. 2138; J. Ravetch, I. Kirsch, P. Leder, *ibid.*, p. 6734.
20. I. Kirsch, J. Ravetch, S.-P. Kwan, E. Max, R. Ney, P. Leder, *Nature (London)* **293**, 585 (1981).
21. T. Maniatis *et al.*, *Cell* **15**, 687 (1978).
22. B. Hohn and K. Murray, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3259 (1977).
23. D. Rimm, D. Horness, J. Kucira, F. Blattner, *Gene* **12**, 301 (1980).
24. W. D. Benton and R. W. Davis, *Science* **196**, 180 (1977).
25. N. Obata *et al.*, *Gene* **9**, 87 (1980).
26. A. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 560 (1977).
27. J. Pink, S. Buttery, G. DeVries, C. Milstein, *Biochem. J.* **117**, 33 (1970).
28. We thank M. Harper for advice and teaching, R. Robinson and J. Battey for helpful mathematical discussions, G. Stetten and S. Latt for sharing Q-banding techniques, and T. Broderick for the preparation of this manuscript.

9 December 1981; revised 5 February 1982

Role of Vitamin B_{12} in Methyl Transfer for Methane Biosynthesis by *Methanosarcina barkeri*

Abstract. *When* Methanosarcina barkeri *is grown on methanol as the sole carbon source, a B_{12}-containing protein is synthesized by this organism. This B_{12} protein contains bound aquocobalamin, and when this cofactor is reduced and methylated with [^{14}C]methyl iodide, the resultant [^{14}C]methyl B_{12} protein is extremely active in the biosynthesis of ^{14}C-labeled methane. These findings indicate that a B_{12}-dependent system is operative in the biological formation of methane in addition to other systems that are B_{12}-independent.*

Methanosarcina barkeri is one of the most metabolically diverse methane producers. In addition to producing methane from hydrogen and carbon dioxide, this organism will grow heterotrophically on methanol, methylamine, or acetate as the carbon source (*1*). The methanol metabolic pathway is

$$4CH_3OH \rightarrow 3CH_4\uparrow + CO_2\uparrow + 2H_2O$$

In 1968, Blaylock (*2*) showed that a B_{12}-containing protein was an essential component in the formation of methane from methanol. This B_{12} protein, which was partially purified from extracts of methanol-grown *Methanosarcina barkeri*, was shown to have a molecular weight of approximately 200,000; in the presence of crude extracts, it was methylated by the growth substrate methanol (*2*). The B_{12} chromophore was characteristic of bound aquocobalamin, with a maximum absorption at 352 nm.

We purified the B_{12} protein from cell extracts of methanol-grown *Methanosarcina barkeri* by elution from DEAE-52 cellulose with 0.25*M* sodium chloride, followed by chromatography on a second DEAE-52 cellulose column with 1*M* tris buffer (*p*H 7.2) as the eluting salt. After concentration by pressure dialysis, the protein was purified to homogeneity

0036-8075/82/0416-0303$01.00/0 Copyright © 1982 AAAS