III regions. The multiplicity of hybridizing fragments in the class I region suggests that the class I genes are dispersed throughout the 1800 to 2000 kb comprising the class I region. In contrast, since no class II beta genes were found in the 340-kb Mlu I fragment that hybridized with DR_α, the class II genes appear to be clustered in the centromeric half of the 920-kb Not I fragment. These data are in accord with those recently determined by Hardy *et al.* (*12*) who have shown that the DO and DX class II subregions also reside between DP and DR and that DZ is closely linked to DP. Thus, there may be as much as 300 kb between the class II and class III subregions in which classical HLA genes are absent. In addition to the class I, II, and III genes, other genes are interspersed in or located near the MHC (*5*). Since the PFG estimation of the extent of HLA exceeds the amount isolated in molecular clones by a factor of at least 2, these data indicate ample room for these and other nonclassical HLA-linked genes or gene families.

This study has examined the application of pulsed field gel electrophoresis and hybridization to the molecular organization of the HLA gene complex. The preparation of essentially intact human DNA suitable for restriction digestion, the identification of restriction enzymes that generate fragments suitable for megabase-scale restriction mapping, and the development of electrophoresis and hybridization procedures that detect fragments throughout a range of 10 to 1000 kilobases demonstrate that the techniques employed are suitable for the efficient analysis of megabase regions of mammalian DNA. The potential now exists for the use of these techniques in conjunction with classical genetics, standard cloning techniques, and chromosome linking and chromosome jumping libraries (*4, 5*), in the construction of an extensive molecular map of the human genome.

Note added in proof: Partial digestion data confirm the placement of the 190-kb Not I fragment adjacent to the 540-kb Not I fragment.

REFERENCES AND NOTES

1. E. B. Robson and L. U. Lamm, *Cytogenet. Cell Genet.* **37**, 47 (1984).
2. R. Srivastava, B. W. Duceman, P. A. Biro, A. K. Sood, S. M. Weissman, *Immunol. Rev.* **84**, 93 (1985).
3. A. Korman *et al.*, *ibid.* **85**, 45 (1985).
4. C. L. Smith *et al.*, *Methods Enzymol.*, in press.
5. S. K. Lawrance *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **51**, 123 (1986).
6. D. C. Schwartz and C. R. Cantor, *Cell* **37**, 67 (1984).
7. C. L. Smith *et al.*, in preparation.
8. A. K. Sood, D. Pereira, S. M. Weissman, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 616 (1981).
9. J. I. Bell *et al.*, *ibid.* **82**, 3405 (1985).
10. P. Gladstone, L. Fueresz, D. Pious, *ibid.* **79**, 1235 (1982).
11. R. Srivastava *et al.*, in preparation.
12. D. A. Hardy, J. I. Bell, E. O. Long, T. Lindsten, H. O. McDevitt, *Nature (London)* **323**, 453 (1986).
13. E. Southern, *J. Mol. Biol.* **98**, 503 (1975).
14. S. K. Lawrance, H. K. Das, J. Pan, S. M. Weissman, *Nucleic Acids Res.* **13**, 7515 (1985).
15. H. K. Das, S. K. Lawrance, S. M. Weissman, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3542 (1983).
16. A. S. Whitehead, G. Goldberger, D. E. Woods, A. F. Markham, H. R. Colten, *ibid.*, p. 5387.
17. B. H. Keller and H. T. Orr, *J. Immunol.* **134**, 2727 (1985).
18. M. C. Carroll, R. D. Campbell, D. R. Bently, R. R. Porter, *Nature (London)* **307**, 237 (1984).
19. R. Srivastava *et al.*, *Trans. Am. Assoc. Physicians*, in press.

20. The authors acknowledge the excellent technical assistance of P. Warburton, J. Economе, and S. Rucker and thank D. Uranowski for preparing the manuscript. Supported by grants from the National Cancer Institute (5-T35-CA-39782) and the Hereditary Disease Foundation.

14 October 1986; accepted 5 January 1987

β Amyloid Gene Duplication in Alzheimer's Disease and Karyotypically Normal Down Syndrome

JEAN-MAURICE DELABAR, DMITRY GOLDGABER, YVON LAMOUR, ANNIE NICOLE, JEAN-LOUIS HURET, JEAN DE GROUCHY, PAUL BROWN, D. CARLETON GAJDUSEK, PIERRE-MARIE SINET

With the recently cloned complementary DNA probe, λAm4 for the chromosome 21 gene encoding brain amyloid polypeptide (β amyloid protein) of Alzheimer's disease, leukocyte DNA from three patients with sporadic Alzheimer's disease and two patients with karyotypically normal Down syndrome was found to contain three copies of this gene. Because a small region of chromosome 21 containing the *ets*-2 gene is duplicated in patients with Alzheimer's disease, as well as in karyotypically normal Down syndrome, duplication of a subsection of the critical segment of chromosome 21 that is duplicated in Down syndrome may be the genetic defect in Alzheimer's disease.

A COMPLEMENTARY DNA (cDNA) probe λAm4 for the chromosome 21 gene encoding the amyloid polypeptide (β amyloid protein) of Alzheimer's disease (AD-AP gene) was recently cloned and sequenced (*1*). The polypeptide encoded by this gene is found in the brains of patients with Alzheimer's disease, as well as in adult patients with Down syndrome (*2*). We present results of gene dosage measurements in leukocyte DNA, using this cDNA as a probe, that show that the AD-AP gene is duplicated in patients with Alzheimer's disease and in patients with karyotypically normal Down syndrome (*3, 4*).

DNA was obtained from leukocytes from seven healthy control subjects (four young adults and three aged adults), five patients with trisomy 21 Down syndrome, two patients with karyotypically normal Down syndrome (and their four normal parents), and three patients with sporadic Alzheimer's disease. The clinical diagnosis of Alzheimer's disease was based on criteria defined by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) (*5*). Preliminary experiments established appropriate autoradiographic exposure times for calibrating dose-response curves for the quantity of DNA as a function of band intensity measured by transmission densitometry. The linear response range of the curves was used in all experiments. An 8.6-kb band, which is recognized by the human AD-AP cDNA probe (λ Am4) located on chromosome 21, and a 4.1-kb reference band, which is recognized by the human proα2 (I) collagen probe (COL1A2) located on chromosome 7 (*6*), are seen on Eco RI–digested DNA from the patients and control subjects (Fig. 1). Actual densitometric measurements were carried out by using autoradiographs. By assuming that the DNA from the control subjects had a gene dosage number of two for both the COL1A2 reference probe and the AD-AP gene, AD-AP gene dosage numbers in the patients were deduced from the ratio of the intensities of the 8.6-kb band to the 4.1-kb reference band. Four additional chromosome 21 probes were used or are referred to in this study: human superoxide dismutase-I (*SOD1*), anonymous DNA sequence D21S11 located proximal to *SOD*I, human proto-oncogene *ets*-2, and the estrogen-inducible sequence expressed in breast cancer BCEI located in 21q22.3 (*7*).

J.-M. Delabar, A. Nicole, P.-M. Sinet, Laboratoire de Biochimie Génétique (Professor Kamoun), J. E. CNRS 34623, Hôpital Necker, 149 rue de Sèvres, 75743 Paris Cedex 15, France.
D. Goldgaber, P. Brown, D. C. Gajdusek, Laboratory of Central Nervous System Studies, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.
Y. Lamour, INSERM U.161, 2 rue d'Alésia, 75014 Paris, France.
J.-L. Huret, CHRU Le Milétrie, 86021 Poitiers, France.
J. de Grouchy, INSERM U.173 and U.A. 119 CNRS, Hôpital Necker, 149 rue de Sèvres, 75743 Paris Cedex 15, France.

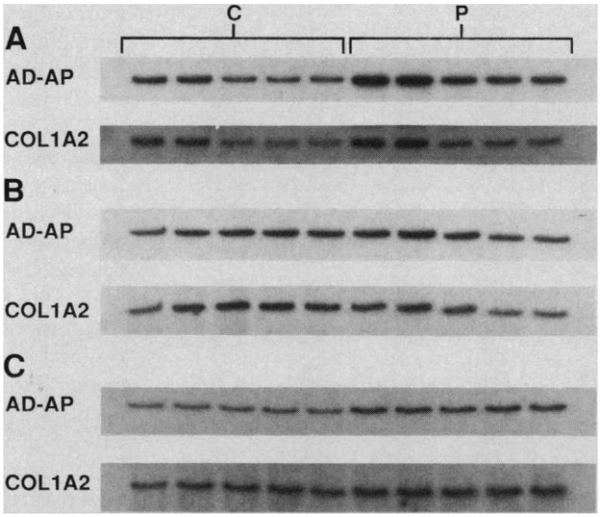

Fig. 1. Photographs of autoradiographs showing DNA sequences (bands) recognized by the AD-AP probe (top) and COL1A2 probe (bottom) in DNA from three patients (P) (five right lanes) and three normal controls (C) (five left lanes). (**A**) Patient with trisomic Down syndrome (47,XY, +21DS); (**B** and **C**) patients with Alzheimer's disease. DNA was purified from leukocytes, digested with Eco RI, size fractionated by electrophoresis in 0.8% agarose gel, and transferred to Zetabind membranes. Prehybridization, hybridization, and washing of the membranes were carried out as described in the Zetabind protocols. Membranes were first hybridized with a ^{32}P-labeled (*12*) COL1A2 probe, and the 4.1-kb DNA sequence recognized by this probe was used as a reference band. The same membrane was then rehybridized with the AD-AP probe, and the 8.6-kb DNA sequence recognized by this probe was used for quantification of gene dosage. Quantification of the DNA sequences recognized by the probes was carried out by transmission densitometry of autoradiographs with a thin-layer chromatography laser scanner (C6-930, Shimadzu). The relation between band intensity and DNA quantity was calibrated by densitometry of DNA dot blot autoradiographs, after preliminary experiments with different exposure times had established the linear response range of the calibration curves.

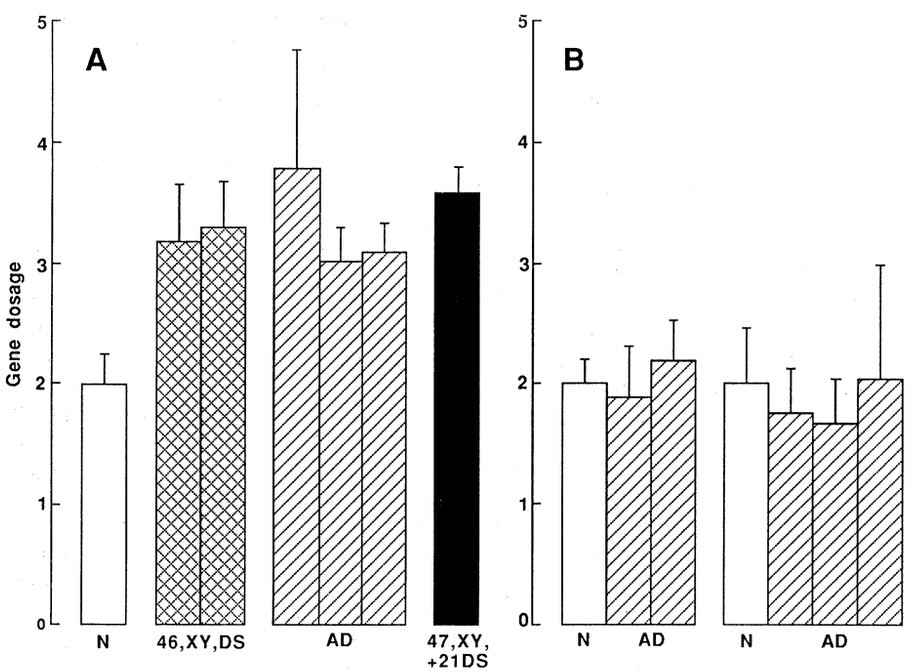

Fig. 2. Gene dosage histograms for (**A**) the AD-AP gene and (**B**) anonymous DNA sequences D21S11 and BCEI. Normal controls (N) included four healthy adults under the age of 40, three adults over the age of 60 free from any neurologic or psychiatric disease, and all four parents of the two patients with karyotypically normal Down syndrome. The two patients with karyotypically normal Down syndrome (46,XY,DS) were 2 and 30 years old; detailed clinical observations are presented elsewhere (*3, 4*). The three female patients with sporadic Alzheimer's disease (AD), aged 67, 77, and 86 years, had Mini-Mental State Examination scores ranging from 0 to 15. Five patients with trisomy 21 Down syndrome (47,XY,+21DS) were also used in this study. Quantification of the DNA specific for each probe was carried out as described in Fig. 1. For each lane of the blots the ratio of intensity of the 8.6-kb AD-AP band versus the intensity of the reference 4.1-kb COL1A2 band was calculated. For each blot, ratio values were standardized by assuming a gene dosage number of two in the DNA from controls, and between 5 and 15 assays were performed for each patient. Data are expressed as mean ± SD. (**A**) AD-AP gene dosage in DNA from karyotypically normal patients with Down syndrome (46,XY,DS, cross-hatched); patients with sporadic Alzheimer's disease (AD, shaded); and trisomy 21 patients with Down syndrome (47,XY,+21DS, black). (**B**) Gene dosage of chromosome 21 anonymous DNA sequences D21S11 (left) and BCEI (right) in patients with Alzheimer's disease (AD) and normal controls (N).

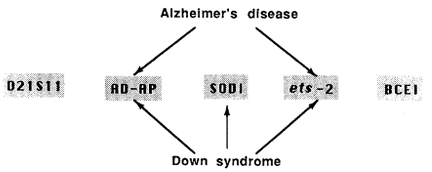

Fig. 3. Schematic representation of duplicated and nonduplicated genes in Alzheimer's disease and in karyotypically normal Down syndrome. Arrows point to the genes that are duplicated in each disease.

In patients with trisomy 21 Down syndrome (47,XY,+21DS), the intensity of the AD-AP gene band was 50% greater than in the normal control subjects. We were, therefore, able to verify that these patients had the expected three copies of the AD-AP gene (Fig. 2A). By using the same approach, we found that three copies of the AD-AP gene were also present in all three patients with Alzheimer's disease (AD) and in both patients with karyotypically normal Down syndrome (46,XY,DS) (Fig. 2A). It has been shown that the *ets*-2 gene is also duplicated in these patients (*8*), that the *SOD*1 gene was duplicated only in the Down syndrome patients (*3, 4, 8*), and that the D21S11 and the BCEI DNA sequences were not duplicated in either group of patients (Fig. 2B). These data are shown in schematic form in Fig. 3. Our results support the hypothesis that Alzheimer's disease involves the duplication of a subsection of the critical segment of chromosome 21 that is duplicated in Down syndrome (*9*), and thus substantiates the existence of a genetic defect in Alzheimer's disease. The exact size and continuity of the subsection and the existence of other aberrations in the genetic material of the involved region are unknown.

It could be argued that this duplication might be postzygotic, because we investigated only one cell type, leukocytes. However, because the phenotypic features of Down syndrome and Alzheimer's disease surely result from involvement of several different cell types, it seems more likely that the rearrangement originates in parental gametes. For example, this gene duplication could be caused by unequal crossing over during meiosis I, or unequal chromatid exchange during meiosis II or premeiotic stages. This particular region of chromosome 21 might represent a "recombination hot spot" that could be due to the presence of repeated DNA sequences, as recently demonstrated for the 15q11 region in Willi-Prader syndrome (*10*).

Because with increasing age, the majority of people will develop neuropathological changes similar to those seen in Alzheimer's

disease, it is reasonable to propose that an excess gene dosage of certain genes, with overproduction of the corresponding proteins (*11*) could cause these changes to occur prematurely. Moreover, even if Alzheimer's disease requires a duplication of a segment of chromosome 21 containing the AD-AP gene, it does not follow that all bearers of this duplication will develop the disease. The roles played by faulty regulatory mechanisms and environmental stress are still poorly understood, but are certainly important.

REFERENCES AND NOTES

1. D. Goldgaber *et al.*, in *Alzheimer's Disease: Advances in Basic Research Therapies*, Proceedings of the Fourth Meeting of the International Study Group on the Pharmacology of Memory Disorders Associated with Aging, Zurich, Switzerland, 16 to 18 January 1987 (Center for Brain Sciences and Metabolism Charitable Trust, Cambridge, MA, 1987), pp. 209–217; D. Goldgaber, M. I. Lerman, O. W. McBride, U. Saffiotti, D. C. Gajdusek, *Science* **235**, 877 (1987).
2. G. G. Glenner and W. C. Wong, *Biochem. Biophys. Res. Commun.* **122**, 1131 (1984); C. L. Masters *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4245 (1985).
3. J.-L. Huret *et al.*, *Hum. Genet.*, in press.
4. J.-M. Delabar *et al.*, in preparation.
5. P. McKhann *et al.*, *Neurology* **34**, 939 (1984).
6. J. C. Myers *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3516 (1981).
7. Human gene mapping 8, *Cytogenet.* **1–4** (1985).
8. J.-M. Delabar *et al.*, *Ann. Genet.* **29**, 226 (1986).
9. M. Schweber, *Ann. N.Y. Acad. Sci.* **450**, 223 (1985).
10. A. Donlon, M. Lalande, A. Wyman, G. Bruns, S. A. Latt, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4408 (1986).
11. P.-M. Sinet, D. Allard, J. Lejeune, H. Jerome, C. R. *Acad. Sci. (Paris)* **278**, 3267 (1974); S. Sichitiu, P.-M. Sinet, J. Lejeune, J. Frezal, *Humangenetik* **23**, 65 (1974); W. W. Feaster, L. W. Kwok, C. J. Epstein, *Am. J. Genet.* **29**, 563 (1977).
12. A. P. Feinberg and B. Vogelstein, *Ann. Biochem.* **132**, 6 (1983); *ibid.* **137**, 266 (1984).

13. Supported in part by the Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale (INSERM). We thank M. Roudier and P. Devous for allowing us to study patients under their care; M. Jerome and P. Kamoun for their helpful encouragement; and F. Ramirez for the gift of the collagen probe.

3 February 1987; accepted 17 February 1987

Transposition of Gram-Positive Transposon Tn*916* in *Acholeplasma laidlawii* and *Mycoplasma pulmonis*

KEVIN DYBVIG AND GAIL H. CASSELL

Mycoplasma genetics has been limited by a lack of genetic tools such as selectable markers, methods to transfer DNA, and suitable vectors for cloning. Studies were undertaken to examine the potential of using the streptococcal transposon Tn*916* as a mycoplasma genetic tool. The *Escherichia coli* plasmid pAM120, which contains Tn*916*, was transformed into *Acholeplasma laidlawii* and *Mycoplasma pulmonis*. Transposition of Tn*916* into the mycoplasma chromosome apparently occurred by an excision-insertion mechanism. This example shows that newly introduced DNA from other bacteria can be successfully expressed in mycoplasma and that Tn*916* should serve as a powerful genetic tool for the study of mycoplasmas.

MYCOPLASMAS ARE WALL-LESS prokaryotes that cause disease in plants, insects, and animals, including man. The five currently recognized genera of mycoplasmas, *Acholeplasma, Spiroplasma, Mycoplasma, Ureaplasma,* and *Anaeroplasma*, are a diverse group of related organisms that have evolved from Gram-positive bacteria (*1*). The development of mycoplasma genetics has been hampered by a lack of selectable markers and a lack of methods to transfer DNA such as conjugation, transformation, and transduction. Therefore, most studies in mycoplasma genetics have been limited to the cloning and subsequent analysis of mycoplasma DNA in *Escherichia coli*; few studies in mycoplasma genetics have involved the isolation and analysis of mutants. In this report we describe the transformation of mycoplasmas with the transposable element Tn*916*. The convenience of insertional mutagenesis through transposition of Tn*916* should stimulate the field of mycoplasma genetics.

Tetracycline-resistant isolates of *Mycoplasma hominis* and *Ureaplasma urealyticum* have DNA sequences homologous to the streptococcal *tetM* determinant (*2, 3*). The *tetM* determinant may have disseminated from streptococci via a conjugative transposon—for example, Tn*916*, a broad–host range transposon with which *tetM* is associated (*4–8*). The plasmid pAM120 (21 kilobases) contains Tn*916* (16 kilobases) cloned into the vector pGL101 in *E. coli* strain DH1 (*8*). We used pAM120 to transform mycoplasmas to determine whether Tn*916* will function in these cells. We were successful in transformation of both *Acholeplasma laidlawii* and the murine pathogen *Mycoplasma pulmonis*.

Transformations were based on the polyethylene glycol (PEG) procedure developed for transfection of *A. laidlawii* with mycoplasma viral DNA (*9*). *Acholeplasma laidlawii* cells were propagated in tryptose broth (*10*) and assayed as colony-forming units (CFU) on mycoplasma agar plates (*11*). Ten micrograms of pAM120 DNA were used to transform 250 μl (about 2×10^7 CFU) of cells from a culture in logarithmic growth phase. After transformation, cells were incubated at 37°C in tryptose broth for 30 minutes to allow for expression of the tetracycline-resistance determinant and then plated onto freshly made agar plates supplemented with tetracycline at a concentration of 2 μg/ml. Selection with tetracycline at a concentration of 4μg/ml gave somewhat more inconsistent transformation frequencies, and incubation for more than 30 minutes before plating did not increase transformation frequencies. The transformation mixtures were assayed on agar plates in incubation at 37°C in the dark (tetracycline is light-sensitive) for 4 days. Colonies were picked by stabbing the plate with a sterile 1-ml pipette and placing the agar plug in 1 ml of tryptose broth. This culture was incubated at 37°C for 2 hours; tetracycline was added to a concentration of 4 μg/ml; and the culture was incubated again for 2 to 3 days until turbidity was observed. If tetracycline was added immediately after picking colonies, some of the cultures did not grow. Waiting 2 hours before adding the tetracycline alleviated this problem (*12*). Once the isolates were actively growing in liquid medium, they could be incubated in medium containing high levels of tetracycline, at least 40 μg/ml, and still retain cell growth.

Transformation of *M. pulmonis* was similar to that of *A. laidlawii*. Mycoplasma medium (*11*), instead of tryptose broth, was used for propagation of *M. pulmonis*. Important for obtaining an efficient protocol for transformation of *M. pulmonis* was the realization that mycoplasma medium and tryptose broth were inhibitory. The first step in the protocol for transformation of *A. laidlawii* is to harvest cells by low-speed centrifugation and resuspend the cell pellets in fresh tryptose broth (*9*). We removed the inhibitory medium from *M. pulmonis* by resuspending the cell pellets in buffer. Examination of various buffers showed that the optimum PEG concentration was dependent on the buffer (*13*). After transformation of *M. pulmonis*, the incubation time allowed for expression of the *tetM* determinant was 2 hours.

Acholeplasma laidlawii was chosen as the recipient for our initial transformation studies because a PEG-mediated transformation procedure had already been developed for transfection of this mycoplasma with viral DNAs. Strain 8195 was chosen because it apparently lacks a restriction and modification system (*14*). We obtained tetracycline-resistant transformants of *A. laidlawii* strain

Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.