es analogous to the one described here occur with other transposable elements, then the proposed excision-loss mechanism could explain unusual patterns in the distribution and abundance of transposable elements among related species (*12*).

REFERENCES AND NOTES

1. J. W. Jacobson, M. M. Medhora, D. L. Hartl, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8684 (1986).
2. B. H. Judd, in *The Genetics and Biology of Drosophila*, M. Ashburner and E. Novitski, Eds. (Academic Press, London, 1976), vol. 1b, pp. 767–799.
3. J. W. Jacobson and D. L. Hartl, *Genetics* **111**, 57 (1985); D. S. Haymer and J. L. Marsh, *Dev. Genet.* **6**, 281 (1986).
4. Rate of reversion to w^+ in females estimated at 1.0×10^{-2} and in males 2.5×10^{-2} ($\chi^2_{(1)} = 32.6$, $P << 0.01$, 9993 X chromosomes tested).
5. J. T. Lis, J. A. Simon, C. A. Sutton, *Cell* **35**, 403 (1983).
6. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
7. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
8. P. W. J. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *ibid.* **113**, 237 (1977).
9. F. A. Laski, D. C. Rio, G. M. Rubin, *Cell* **44**, 7 (1986).
10. N. V. Federoff, in *Mobile Genetic Elements*, J. A. Shapiro, Ed. (Academic Press, New York, 1983), pp. 1–63.
11. S. W. Emmons and L. Yesner, *Cell* **36**, 599 (1984); D. Eide and P. Anderson, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1756 (1985); D. G. Moerman, G. M. Benian, R. H. Waterston, *ibid.* **83**, 2579 (1986); S. W. Emmons, S. Roberts, K. Ruan, *Mol. Gen. Genet.* **202**, 410 (1986).
12. A. P. Dowsett and M. W. Young, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4570 (1982).

13. We thank M. Medhora, K. Maruyama, D. Moerman, D. Garza, S. Lawler, R. Francis, and R. DeSalle for helpful conversations; A. MacPeek for technical assistance; and R. F. Lyman for providing the *D. mauritiana white* strain. Special thanks to J. A. Coyne for providing the *D. simulans forked, net plum, scarlet ebony* strain. G.J.B. acknowledges the financial support of the Fulbright Commission. This work was supported by National Institute of General Medical Sciences grant GM33741.

17 October 1986; accepted 16 January 1987

Human Lipoprotein Lipase Complementary DNA Sequence

KAREN L. WION, TODD G. KIRCHGESSNER, ALDONS J. LUSIS, MICHAEL C. SCHOTZ, RICHARD M. LAWN*

Lipoprotein lipase is a key enzyme of lipid metabolism that acts to hydrolyze triglycerides, providing free fatty acids for cells and affecting the maturation of circulating lipoproteins. It has been proposed that the enzyme plays a role in the development of obesity and atherosclerosis. The human enzyme has been difficult to purify and its protein sequence was heretofore undetermined. A complementary DNA for human lipoprotein lipase that codes for a mature protein of 448 amino acids has now been cloned and sequenced. Analysis of the sequence indicates that human lipoprotein lipase, hepatic lipase, and pancreatic lipase are members of a gene family. Two distinct species of lipoprotein lipase messenger RNA that arise from alternative sites of 3′-terminal polyadenylation were detected in several different tissues.

LIPOPROTEIN LIPASE (LPL) PLAYS A major role in the regulation of lipid metabolism. Its primary function is the hydrolysis of the core triglycerides of circulating chylomicrons and very low density lipoproteins (VLDL). In a broader sense, LPL affects the maturation of several classes of lipoprotein particles and may facilitate the transfer of cholesteryl esters to endothelial cells. Thus, the fate of plasma cholesterol, as well as triglycerides, depends on the proper function of LPL. Lipoprotein lipase is synthesized in parenchymal cells of many tissues, but functions on the luminal surface of vascular endothelium, where it is anchored by a membrane-bound glycosaminoglycan chain. In the presence of the cofactor apoliprotein C-II, LPL hydrolyzes dietary or endogenous triglycerides to monoglycerides and free fatty acids, which are taken up by cells for oxidation (muscles) or storage (adipose). The enzymatic activity is regulated by nutrients and hormones to allow tissues to respond to energy requirements and storage needs (*1*). To obtain new insight into structural, regulatory, and genetic features of LPL, we have cloned and sequenced LPL complementary DNA (cDNA) derived from human adipose tissue.

LPL clones from a mouse macrophage cDNA library were identified by screening with antibodies to bovine milk LPL (*2*). We used a 500–base pair (bp) mouse partial cDNA clone to identify clones from a human adipose tissue cDNA library (*3*). Sequence analysis of overlapping cDNA clones yielded the DNA and predicted protein sequence of the 448–amino acid LPL protein, which is preceded by a 27-residue prepeptide (Fig. 1).

The human LPL cDNA clones that were initially sequenced (designated LPL35 and LPL37) contain 174 bp of 5′ and 1556 bp of 3′ untranslated sequence and contain 3155 bp overall. The poly(A) tail is preceded by the common polyadenylation signal AATAAA. However, when cloned LPL cDNA was radiolabeled and hybridized to poly(A)$^+$ RNA derived from several human tissues, two bands of roughly equal intensity were seen in each positive tissue (Fig. 2). The two RNA species are approximately 3350 and 3750 nucleotides in length. The LPL messenger RNA (mRNA) is seen most abundantly in adipose and adrenal tissues, in moderate intensity in kidney and intestine, and in barely detectable amounts in pancreas and placenta. We did not detect LPL mRNA in liver, HepG2 (hepatocyte) cells, or white blood cells.

The two different sized RNA species might be due to alternative sites of polyadenylation, splicing, or transcription initiation of the RNA. Several experiments were undertaken to address these possibilities. Oligonucleotide-primed cDNA synthesis of adipose mRNA suggested that only one 5′ end of the message occurs at about 175 nucleotides upstream of the translation initiation codon. This is about 39 nucleotides farther 5′ than the beginning of the cDNA sequence shown in Fig. 1. To explore the possibility of alternative splicing, which could affect the protein coding portions of the mRNA, we subjected nine independent LPL cDNA clones to restriction enzyme analysis. The only differences seen could be attributed to the clones being of various lengths. The issue was resolved by the eventual isolation of clones that extended farther in the 3′ direction. The original cDNA library was rescreened with a 210-bp restriction fragment derived from the 3′ terminus of clone LPL37. This fragment was used as a probe because eight of the nine originally characterized LPL clones were found to terminate within or near a stretch of ten adenosine residues contained within the 3′ untranslated region. This oligo(A) stretch is not a true poly(A) tail; no polyadenylation signal sequence precedes it and clone LPL37 extends through this region and terminates farther downstream in a poly(A) tail. This

K. L. Wion and R. M. Lawn, Department of Cardiovascular Research, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080.
T. G. Kirchgessner and A. J. Lusis, Departments of Medicine and Microbiology, University of California, Los Angeles, CA 90024.
M. C. Schotz, Research, Veterans Administration, Wadsworth Medical Center, Los Angeles, CA 90073, and Departments of Medicine and Microbiology, University of California, Los Angeles, CA 90024.

*To whom reprint requests should be addressed.

sequence apparently serves as a site for oligo(dT) priming of reverse transcriptase, and is thus an impediment to extended synthesis of cDNA clones that begin at the true poly(A) tail or tails. Screening with the restriction fragment probe yielded numerous clones containing the 3′ region of LPL cDNA. The sequence of clone LPL46 overlaps the sequence of LPL37 but is polyadenylated 395 nucleotides farther downstream. The polyadenylation sequence AATAAA also precedes this alternative site of polyadenylation (Fig. 1). These results demonstrate that the two LPL hybridizing mRNAs of roughly 3350 and 3750 nucleotides represent LPL message terminating at roughly equal frequency at polyadenylation sites separated by 395 nucleotides. Hence, the total cDNA sequence of 3550 bp shown in Fig. 1 represents all but approximately 39 nucleotides of 5′ untranslated sequence of the two mRNA species, plus poly(A) tails of several hundred nucleotides located at either one of two alternative 3′ termini.

The first ATG of the cDNA clone initiates an open reading frame coding for a 475–amino acid protein. The amino terminus of the mature protein was determined by comparison of the cDNA sequence with 19 residues of amino-terminal protein sequence of human LPL purified from plasma treated with heparin (*4*). The human protein sequence begins with Ala-Asp-Gln and contains 18 of 19 residues that agree with the cDNA sequence. The mature amino terminus is preceded by an apparent signal peptide of 27 amino acids. This prepeptide consists of the typical hydrophobic core bounded by charged residues, which is cleaved from the mature protein after the second of three consecutive alanine residues. The amino-terminal residues and the sequence of ten different tryptic peptides of bovine milk LPL have been reported (*5*). The amino termini of human and bovine milk LPL have diverged more than the rest of the molecule. The first five residues of the human mature protein sequence do not resemble the amino-terminal residues of bovine LPL. Thereafter, residues 6 to 17 of human LPL can be aligned with residues 8 to 19 of bovine LPL with only one mismatch. Overall, bovine and human LPL show almost total sequence conservation. Of the 139 amino acids identified in the bovine tryptic fragments (excluding the amino terminus), 134 (96%) are identical with their counterparts in the human protein. Presumably this reflects strong evolutionary pressure to maintain the structure of the enzyme.

The human cDNA sequence predicts a translated molecular weight of 50,394 for the mature protein. If one takes into account the estimated carbohydrate content, this is

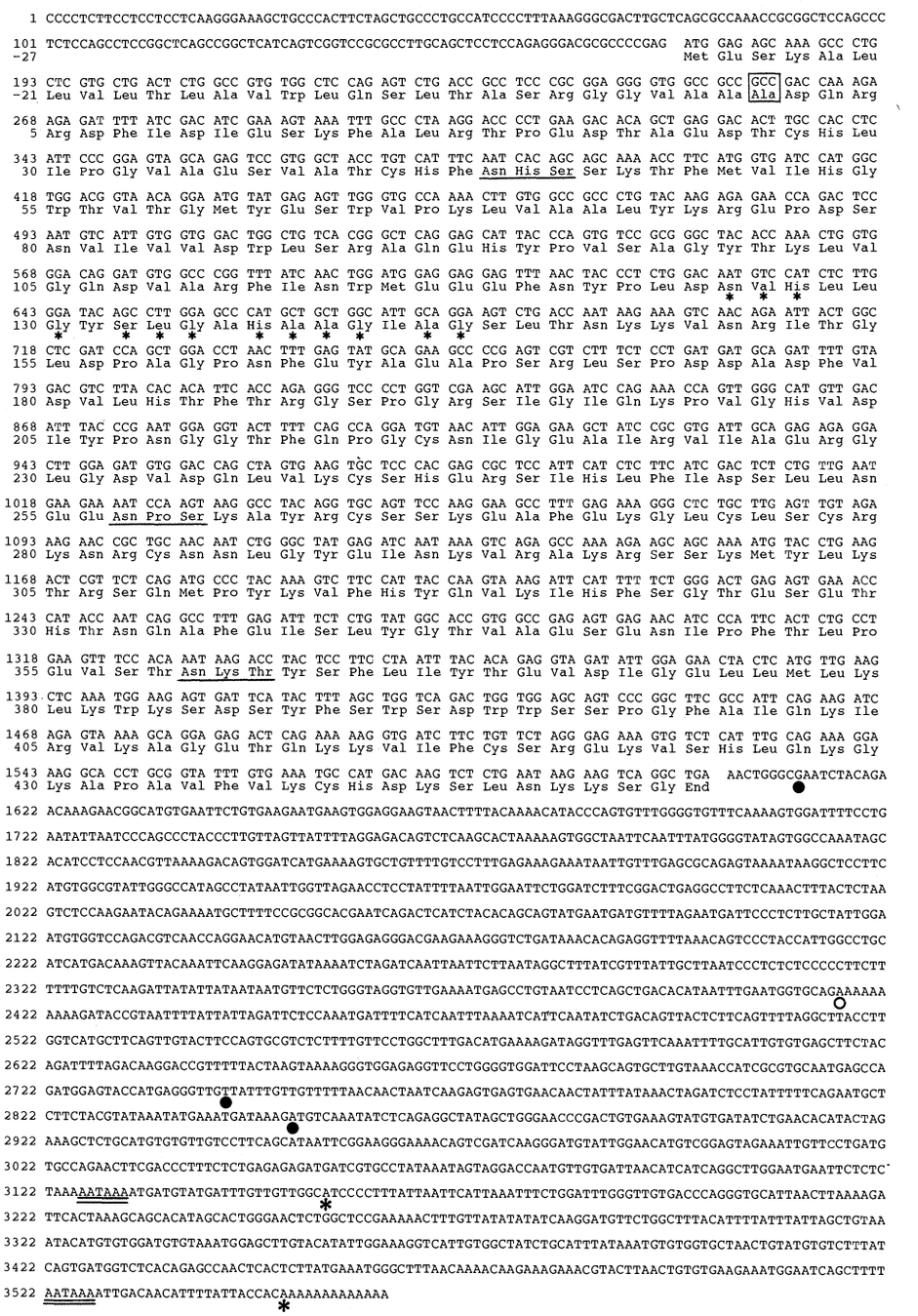

Fig. 1. DNA sequence analysis of human lipoprotein lipase cDNA. DNA sequence was determined by dideoxy chain termination methods (*14*). Nucleotides (left of each row) are numbered from the 5′ terminus of the cDNA clone LPL35. The complete predicted amino acid sequence of LPL is shown below the DNA sequence. Negative amino acid numbers refer to the predicted leader prepeptide; positive numbers refer to the mature protein. The mature amino terminus (boxed in) is positioned by agreement with 19 amino-terminal residues of human LPL reported by Bengtsson-Olivecrona and colleagues (*4*). The predicted *N*-linked glycosylation sites are underlined and amino acids in common with the interfacial binding site of porcine pancreatic lipase are marked below by an asterisk. Double underlines indicate the conserved poly(A) signal hexanucleotide that precedes the two alternative sites of polyadenylation, which occur at nucleotides 3155 and 3550 (indicated by asterisks). Two sequenced clones contained poly(A) tails at nucleotide 3155. In contrast, clone LPL46 has a single A residue at position 3155 and extends downstream to a poly(A) tail at 3550. The sequence shown is the composite of overlapping clones LPL35, LPL37, and LPL46. Three apparent sequence polymorphisms were discovered in the 3′ untranslated region: the nucleotides marked by closed circles are A rather than G (nucleotide 1611), C rather than T (2743), and G rather than A (2851). As discussed in the text, many cDNA clones terminated in the region of ten consecutive A residues in the 3′ untranslated region, which is marked by an open circle.

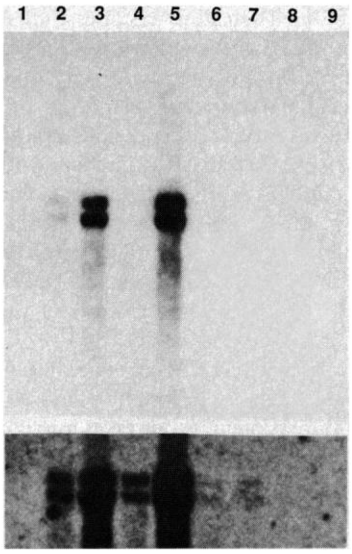

Fig. 2. RNA Northern blot hybridization. Poly(A)$^+$ mRNA (5 μg) was isolated from frozen human tissue or cell samples and subjected to electrophoresis in a 1% agarose, 6% formaldehyde gel, transferred to nitrocellulose, and hybridized with ^{32}P-labeled LPL cDNA clone LPL35 in 5× SSC, 50 m*M* sodium phosphate (*p*H 6.8), 5× Denhardt's solution, 50% formamide, 10% dextran sulfate, and 200 μg/ml of boiled sonicated salmon sperm DNA at 42°C. The filter was then washed in 0.2× SSC containing 0.1% SDS at 65°C and exposed to x-ray film for 8 hours (*15*). RNA sources are: lane 1, liver; 2, intestine; 3, adrenal; 4, kidney; 5, adipose; 6, pancreas; 7, placenta; 8, white blood cells; 9, HepG2 (hepatocyte) cells. In all six tissues where LPL mRNA is present, two hybridizing species of about 3750 and 3350 nucleotides appear in roughly equimolar amounts. The inset at the bottom shows a portion of the same filter exposed for 40 hours in order to visualize the weak signal in lanes 6 and 7 and to further emphasize the negative lanes 1, 8, and 9.

consistent with reports of apparent molecular weights of human LPL of approximately 60,000 (*1*). There are three potential asparagine-linked glycosylation sites (Asn-X-Ser; Asn-X-Thr) in the LPL sequence. The amino acid content of the DNA-derived sequence falls within the range of direct amino acid composition data obtained by four different groups (*6*). Only the predicted number of cysteine and tryptophan residues differ markedly from the one group that determined these particular amino acids. No significant homology could be found in the human LPL sequence to the purported active site peptide of bovine milk LPL presented by Reddy and colleagues (*7*). It is unlikely that this can be accounted for by divergence of bovine and human LPL, given the high degree of sequence conservation.

This study places lipoprotein lipase firmly in a gene family that includes hepatic and pancreatic lipases. Hepatic lipase has many properties in common with LPL, including its location on vascular surfaces. However, it lacks the apolipoprotein C-II cofactor requirement of LPL and may, in fact, be inhibited by apo C-II (*8*). Pancreatic lipase is an acid-stable enzyme that requires a protein cofactor, colipase, for maximal activity. As shown in Fig. 3, the cDNA-derived protein sequences of human LPL and rat hepatic lipase (*9*) have 46% homology. Human LPL and porcine pancreatic lipase are 28% homologous. If one considers the divergence due to different species of origin, the protein similarities are very dramatic. Comparison of the sequences of these related lipases suggests which portions of the protein are responsible for both shared and unique functions such as catalysis and substrate, heparin, and cofactor binding sites. Structural features already established for related proteins can be inferred to apply to LPL if local homology exists, and thus direct the course of experimental confirmation. For example, the interfacial lipid-binding region of porcine pancreatic lipase has been identified (*10*). The sequences are extremely homologous in this region. Thirteen of 18 residues surrounding the "essential serine" of the interfacial binding site of porcine pancreatic lipase are identical to human LPL (these identical residues are marked by asterisks in Fig. 1). Homology to this region has also been noted in hepatic lipase, rat lingual lipase, and human lecithin-cholesterol acyltransferase (*9, 11*).

Computer analysis of the protein sequence predicts certain features of overall structure. The algorithm of Kyte and Doolittle (*12*) yields a moderately hydrophobic index of hydropathy equal to −0.42. The interfacial binding site discussed above lies in one of several regions of particularly hydrophobic nature. Application of a protein structure algorithm (*13*) predicts an α-helix content of about 40%. None of the predicted helical stretches are of obvious amphipathic nature, which would be indicative of domains of interaction with the phospholipid surface of lipoprotein particles. The availability of cloned lipoprotein lipase cDNA will allow analysis of the defect responsible for LPL deficiency in certain subjects and facilitate elucidation of the structure and function of this vital enzyme of lipid metabolism.

```
                               10        20        30
hLPL                   ADQRRDFIDIESKFALRTPEDTAEDTCHLIPGVAE
                          *      *  * *          * * *   *
rHL     GQGVGTEPFGRNLGATEERKPLQKPEIRFLL-FKDESDRLGCQLRPQHPE
                10        20        30        40

           40        50        60        70        80
hLPL    SVATCHFNHSSKTFMVIHGWTVTGMYESWVPKLVAALYKREPDS-NVIVV
            * ** *    * **** * *  * *  * * **  *     **  *
rHL     TLQECGFNSSHPLVMIIHGWSVDGLLETWIWKIVGALKSRQSQPVNVGLV
       50        60        70        80        90

            90       100       110       120       130
hLPL    DWLSRAQEHYPVSAGYTKLVGQDVARFINWMEEEFNYPLDNVHLLGYSLG
        ** * *  **      *  *** **    * **        *** *****
rHL     DWISLAYQHYAIAVRNTRVVGQEVAALLLWLEESMKFSRSKVHLIGYSLG
      100       110       120       130       140

           140         150       160       170       180
hLPL    AHAAGIAGSL--TNKKVNRITGLDPAGPNFEYAEAPSRLSPDDADFVDVL
        **  * ***      *  ********** **      ******* ***
rHL     AHVSGFAGSSMGGKRKIGRITGLDPAGPMFEGTSPNERLSPDDANFVDAI
      150       160       170       180       190

             190       200       210       220       230
hLPL    HTFTRGSPGRSIGIQKPVGHVDIYPNGGTFQPGCNIGEAIRVIAERGLGD
        *****   * * **  *  * * ***** *****   *    *** **
rHL     HTFTREHMGLSVGIKQPIAHYDFYPNGGSFQPGCHFLELYKHIAEHGLNA
      200       210       220       230       240

             240       250       260       270       280
hLPL    VDQLVKCSHERSIHLFIDSLLNEENPSKAYRCSSKEAFEKGLCLSCRKNR
          *  ** **** *******           **    *  **** * * *
rHL     ITQTIKCAHERSVHLFIDSLQHSNLQNTGFHCSNMDTFSQGLCLNCKKGR
      250       260       270       280       290

             290       300       310       320       330
hLPL    CNNLGYEINKVRAKRSSKMYLKTRSQMPYKVFHYQVKIHFSGTESETHTN
        ** *** *       *    * ** * * ** *** ** *     *
rHL     CNSLGYDIRRIGHAKSKTLFLITRAQSPFKVYHYQFKIQFI-NQMEKPIE
      300       310       320       330       340

             340       350        360       370       380
hLPL    QAFEISLYGTVAESENIPFTLPE-VSTNKTYSFLIYTEVDIGELLMLKLK
          *   * **  *   ** ** *    ***** **    ***** *** *
rHL     PTFTMTLLGTKEEIKKIPITLGEGITSNKTYSLLITLDKDIGELIMLKFK
       350       360       370       380       390

                390             400          410       420
hLPL    WKSDSYFS--WSD------WWSSPGFA---IQKIRVKAGETQKKVIFCSR
        *         *        *   *  *      * *******    **
rHL     WENSAVWANVWNTVQTIMLWDTEPHYAGLILKTIWVKAGETQQRMTFCP-
       400       410       420       430       440

                430       440
hLPL    EKVSHLQ-KGKAPAVFVKCHDKSLNKKSG
          *  **       *****  **
rHL     DNVDDLQLHPTQEKVFVKCDLKSKD
        450       460       470
```

Fig. 3. Comparison of lipoprotein lipase and hepatic lipase. The top line presents the amino acid sequence of the mature human LPL protein derived from cDNA sequencing, and the bottom line is the cDNA-derived sequence of rat hepatic lipase reported by Komaromy and Schotz (*9*). Asterisks indicate conserved amino acids. The predicted *N*-linked glycosylation sites of LPL are double underlined and the region of homology to the interfacial binding site of porcine pancreatic lipase is underlined. The overall amino acid homology is 46%. A similar pairwise comparison of human LPL and porcine pancreatic lipase reveals 28% homology.

REFERENCES AND NOTES

1. L. C. Smith and H. J. Pownall, in *Lipases*, B. Borgström and H. L. Brockman, Eds. (Elsevier, Amsterdam, 1984), p. 263; E. A. Nikkilä, in *Metabolic Basis of Inherited Disease*, J. Stanbury, J. W. Wyngaarden, D. Fredrickson, J. Goldstein, M. Brown, Eds. (McGraw-Hill, New York, ed. 5, 1983), p. 622; A. S. Garfinkel and M. C. Schotz, in *Lipoproteins*, A. M. Gotto, Jr., Ed. (Elsevier, Amsterdam, in press).
2. T. Kirchgessner *et al.*, *J. Biol. Chem.*, in press.
3. Frozen human adipose tissue samples were pooled from seven individuals from which RNA was pre-

pared as described by G. Cathala *et al.* [*DNA (N.Y.)* **2**, 329 (1983)]. A cDNA library in λgt10 was prepared and ~700,000 clones were plated and screened without amplification [T. Huynh, R. Young, R. Davis, in *DNA Cloning, A Practical Approach*, D. Glover, Ed. (IRL, Oxford, 1985), vol. 1, p. 49]. A 500-bp fragment of mouse LPL cDNA was ^{32}P-labeled and hybridized with duplicate nitrocellulose filters in 5× SSC (standard saline citrate), 50 m*M* sodium phosphate (*p*H 6.8), 5× Denhardt's solution, 20% formamide, 10% dextran sulfate, and 20 μg/ml of boiled, sonicated salmon sperm DNA at 42°C. The filters were then washed in 2× SSC and 0.1% SDS at 55°C, and exposed to x-ray film. About 180 duplicate positive clones appeared from which DNA of nine clones was purified for detailed analysis.
4. G. Bengtsson-Olivecrona, T. Olivecrona, H. Jörnvall, *Eur. J. Biochem.* **161**, 281 (1986).
5. C. M. Ben-Avram *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4185 (1986).
6. Tabulated in R. Hayashi, S. Tajima, A. Yamamoto, *J. Biochem.* **100**, 319 (1986).
7. M. N. Reddy *et al.*, *J. Biol. Chem.* **261**, 9678 (1986).
8. P. K. J. Kinnunen, in *Lipases*, B. Borgström and H. L. Brockman, Eds. (Elsevier, Amsterdam, 1984), p. 307.
9. M. C. Komaromy and M. C. Schotz, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
10. J. De Caro *et al.*, *Biochim. Biophys. Acta* **671**, 129 (1981); A. Guidoni, F. Benkouka, J. De Caro, M. Rovery, *ibid.* **660**, 148 (1981).
11. A. Docherty *et al.*, *Nucleic Acids Res.* **13**, 1891 (1985); J. McLean *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2335 (1986).
12. J. Kyte and R. F. Doolittle, *J. Mol. Biol.* **157**, 105 (1982).
13. J. Garnier *et al.*, *ibid.* **120**, 97 (1978).
14. J. Messing, R. Crea, P. H. Seeburg, *Nucleic Acids Res.* **9**, 309 (1981); F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
15. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Biology: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 202–203.
16. We thank T. Olivecrona for communication of unpublished data, P. Kern for obtaining adipose tissue, and D. Drayna for initial contributions to this project. This research was supported by Genentech, Inc., NIH grant HL28481, the American Heart Association, Greater Los Angeles Affiliate (grant 492-TG13), and the Veterans Administration. A.J.L. is an established investigator of the American Heart Association.

10 December 1986; accepted 17 February 1987

Ubiquitin Is a Component of Paired Helical Filaments in Alzheimer's Disease

HIROSHI MORI, JUN KONDO, YASUO IHARA

Paired helical filaments (PHF), which constitute a distinct type of pathological neuronal fiber, are the principal constituent of neurofibrillary tangles that occur in the brain of patients with Alzheimer's disease. Their insolubility in sodium dodecyl sulfate and urea has prevented the analysis of their subunit composition by gel electrophoresis. A monoclonal antibody (DF2) was isolated that specifically labeled PHF at both the light and electron microscopic levels. It labeled a small polypeptide (5 kilodaltons) that was shown to be ubiquitin in immunoblots of the soluble fraction of brain homogenates. To obtain direct evidence that ubiquitin is a component of PHF, PHF were treated with concentrated formic acid and digested with lysylendopeptidase; ubiquitin-derived peptides were then identified by reversed-phase high-performance liquid chromatography. Two fragments in the PHF digest were identified as derived from ubiquitin by protein sequencing. This procedure should make possible definitive identification of other PHF components.

THE PROGRESSIVE FORMATION OF unusual neuronal fibers characterizes Alzheimer's disease (AD), which is the most common cause of intellectual failure in aged humans. These fibers are composed of two 10-nm filaments wound into a helix with a half-periodicity of 80 nm and are thus termed paired helical filaments (PHF) (*1*). They accumulate as large perikaryal masses, called neurofibrillary tangles (NFT), or occur as small bundles in dystrophic neurites that form plaques in AD brain. The nature of PHF currently draws much attention because of their unusual morphology, their apparent specificity to human brain, and a possible correlation between their concentration in the brain and the degree of dementia (*2*).

The first step to understanding the origin and nature of PHF should be the identification of their components, but available data conflict. This fact is attributable to the insolubility of PHF (*3*), which has prevented the application of conventional analytical methods. Another approach has been to search for normal soluble proteins, possible precursors of the PHF, that are immunoreactive with polyclonal antibodies to PHF (*4*). This approach has led to the immunochemical identification of the microtubule-associated phosphoprotein, tau, as the major antigenic determinant of PHF (*5*). By means of a similar strategy, we have characterized one monoclonal antibody to PHF whose specificity differs from that of polyclonal PHF antibodies and have found that it recognizes ubiquitin. Furthermore, we have developed a new procedure to digest PHF proteolytically and identified ubiquitin-derived fragments by protein sequencing. This approach will be generally applicable to the analysis of other components of PHF.

PHF were purified in the presence of sodium dodecyl sulfate (SDS) according to a previous method, with minor modifications (*4*); the resultant fractions are referred to as SDS-PHF. SDS-PHF were emulsified with complete or incomplete Freund's adjuvant and injected into a Lewis rat. Three days after the last intravenous injection, rat spleen cells were fused with Sp 2/0-Ag14 mouse myeloma cells. Within eleven 96-well plates, 276 wells were positive by enzyme-linked immunosorbent assay (ELISA) for cells secreting rat immunoglobulin. The second screening for PHF-reactive antibodies with sonicated PHF in an ELISA gave 43 positive wells. Of these 43 wells, 3 wells showed strong reactivities with NFT. After cloning by limiting dilution, one hybridoma (DF2) was established. DF2 monoclonal antibody was of the immunoglobulin M class by the Ouchterlony double-diffusion test.

We have confirmed specific binding of DF2 to PHF in several ways. (i) DF2 stained virtually all isolated NFT prepared under nondenaturing conditions (*4*). The numerous very small tangles observed after staining with DF2 resembled those stained with the polyclonal antibodies to PHF (Fig. 1A). (ii) Tangles isolated by extensive extraction with SDS/ME (2-mercaptoethanol) (*4*) were also intensely stained with DF2, suggesting that a DF2-defined epitope is tightly bound to PHF and not dissociated in SDS. (iii) DF2 decorated PHF, as shown by immunoelectron microscopy (Fig. 1B). Immunogold particles conjugated with DF2 were observed on PHF, but not on such contaminating elements in the PHF fractions as collagen fibers, membranous structures, or other organelles. (iv) DF2 stained both neurofibrillary tangles and senile plaque neurites in formalin-fixed AD brain sections; fine neurites in the cerebral cortex were also immunostained (Fig. 1C). However, unlike polyclonal PHF antibodies (*4*), DF2 definitely stained the background of the tissue sections in both AD and normal brains. The absence of such background staining in the isolated NFT preparations could be ascribed to a lack of soluble DF2-immunoreactive proteins since these tangles were obtained by sedimentation through a sucrose layer (*4*). These observations sug-

H. Mori and Y. Ihara, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173, Japan.
J. Kondo, Mitsubishi Chemical Industries Limited Research Center, Biosciences Laboratory, Kamoshida, Midori-ku, Yokohama 227, Japan.