Vol. 164, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1989 Pages 453-460

CLONING AND COMPARATIVE MAPPING OF A HUMAN CLASS III (χ) ALCOHOL DEHYDROGENASE cDNA

P. RATHNA GIRI, JANE F. KRUG, CHRISTINE KOZAK**, TAMYRA MORETTI, STEPHEN J. O'BRIEN*, HECTOR N. SEUANEZ*, AND DAVID GOLDMAN***

Laboratory on Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Bldg. 10, Rm 3C215, Bethesda, MD 20892

* Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD

** National Institute of Allergy and Infectious Diseases, Bethesda ,MD

Received August 30, 1989

A cDNA encoding human class III (χ, ADH5) alcohol dehydrogenase was isolated, sequenced and used to comparatively map this unusual ADH. In their coding sequences, the three major ADH classes were approximately equisimilar, class II and III ADHs sharing the highest sequence identity (67%). A class III-like ADH was mapped to mouse chromosome 3, site of the ADH gene complex, and synteny of ADH5 with four other ADH loci on human chromosome 4 was confirmed. The nearly full-length 1613 nucleotide cDNA contained 433 nucleotides of 3' nontranslated sequence and two possible initiation sites for translation. A protein of 374 amino acid residues could be synthesized using the potential initiation codon at nucleotide 59. However, use of the likely initiation codon at nucleotide 5 would produce a protein of 392 residues with 19 additional N-terminal residues as compared to the known protein sequence. The derived protein sequence also differs at residue 166, where Tyr is found. This difference, due to a single base substitution, could result from cloning artifact, polymorphism, or two expressed class III ADH genes.

The human alcohol dehydrogenases (ADH: alcohol: NAD^+oxidoreductase) comprise a diverse group of five zinc metalloenzymes which have been assembled into three classes on the basis of sequence and function (1). The three class I ADHs (α, β and γ) are mainly expressed in the liver and associate into homo- and heterodimers. The class II ADH subunit (π) is synthesized in liver and/or stomach (2) and despite a higher K_m for ethanol (3), also plays a role in the metabolism of ethanol (4). Unlike these four ADHs, class III has a very low affinity for ethanol (5), has been found in all tissues examined (6) and does not have a well established physiologic role, although it is likely to be involved in the metabolism of long chain alcohols and ω-hydroxyfatty acids (7).

Determination of the protein and DNA sequences of class I and II ADHs (8,9) and sequencing of the class III ADH protein led to the realization that the ADH classes are approximately equidistant in terms of coding sequence identity, but it was also proposed that class III ADH, because of its resemblance to plant ADHs, is the most ancient of the mammalian ADH

*** Corresponding author.

0006-291X/89 $1.50

clan (10). The relationship of class III ADH to the other ADH classes can be more precisely addressed using the cloned gene. A second question is whether ADH5 resides in a gene complex. The five human ADHs have been localized to the region 4q21-25 and in the mouse, four ADH structural and regulatory genes map to chromosome 3. However, the location of mouse class III ADH is unknown. To improve our understanding of the genomic position, structure and evolution of class III ADH, we report here the molecular cloning and sequence of its cDNA and the comparative chromosomal mapping of the class III ADH locus in mouse and human.

MATERIALS AND METHODS

Isolation and sequencing of cDNA clones: Class III ADH was purified from human liver according to Wagner *et al.* (11) with a final anion exchange chromatography step. The purified active enzyme revealed a single band when separated by 10% SDS PAGE and stained with silver. The N-terminus was found to be blocked, as reported (10). Therefore, the protein was digested with Lys-specific endopeptidase (Boehringer Mannheim), and peptides were purified using reverse-phase high performance liquid chromatography (10). Eight peptides representing approximately 40% of the complete class III ADH were sequenced. The protein sequence obtained was in agreement with the full class III ADH protein sequence later reported by Kaiser *et al.* (10).

Two mixed oligonucleotides were synthesized, 5' end-labeled (12) and used to screen a human liver cDNA library constructed in lambda gt 11. Probe A corresponded to amino acids 85-95 and probe B to amino acids 347-357. Plaques were screened by the method of Hanes and Higgins (13), and positively hybridizing phages were plaque-purified to homogeneity. The cDNA inserts were ligated into the *Eco* RI site of vector pMLB 1113 (14). DNA was sequenced by the dideoxynucleotide method of Sanger *et al.* (15) using either the system developed by Messing and Vieira (16) and modified by Zagursky and Berman (14) or by the method for using double-stranded DNA as modified by Zagursky *et al.* (17). Nucleotides were labeled with [^{35}S] dATP (Amersham, approximately 300 Ci/m mol). Sequenase (United States Biochemical Corporation) reagents were employed and reaction products were separated on ultrathin wedge gels prepared with 7% acrylamide and 8M urea. All sequences were analyzed on both DNA strands and on overlapping fragments. For restriction mapping, enzyme digestions were performed as recommended by the manufacturer, and DNA fragments were resolved by electrophoresis on 0.8% agarose gels. For DNA probe analyses, cDNA probes were labeled by the random primer method and oligonucleotide probes were end-labeled.

Extraction of RNA from tissues and RNA blot hybridization: RNA was extracted by the procedure of Cathala *et al.* (18), and poly (A)+ enriched RNA was prepared by oligo(dT)-cellulose column chromatography (19). Ethanol-precipitated RNA was dissolved in a solution of 50% formamide, 20%formaldehyde, 1 mM EDTA, 150 mM sodium acetate and 20 mM MOPS, denatured by heating to 65°C for 5 min, fractionated on 1% formaldehyde agarose gels, electrotransferred to Gene Screen nylon membrane (NEN) and hybridized following the procedure of Chen-Kiang *et al.* (20). The blots were prehybridized 2 hrs with 50% formamide, 5xSSC (1xSSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7), 20 mM NaH_2PO_4, pH 6.5, yeast tRNA (250 ug/ml), herring sperm DNA (250 ug/ml), 5x Denhardt's solution and 0.1% SDS. The hybridization was carried out with prehybridization buffer: dextran sulphate (4:1) with 1 x 10^6 cpm /ml of nick-translated ADH5-14.1 cDNA. Following hybidization, RNA blots were washed twice with 2xSSC for 10 min at room temperature and with 0.1xSSC, 0.1% SDS for 30 min at 65°C. Blots were then used to expose X-ray film. Hybridized probes were removed with 96% formamide, 10 mM EDTA , and 10 mM Tris-HCl and blots were rehybridized with a class I ADH cDNA (ADH2) and class II-specific oligomers.

RESULTS

Isolation of cDNA clones ADH5-14.1 and ADH5-30.1: Two mixed 33-mer oligonucleotides [A, corresponding to nucleotides 256-288, and B, corresponding to nucleotides 1042-1074] were used in colony hybridization screening of a human liver cDNA library. Five of 50,000 plaques screened gave positive hybridization signals. After successive screenings, two

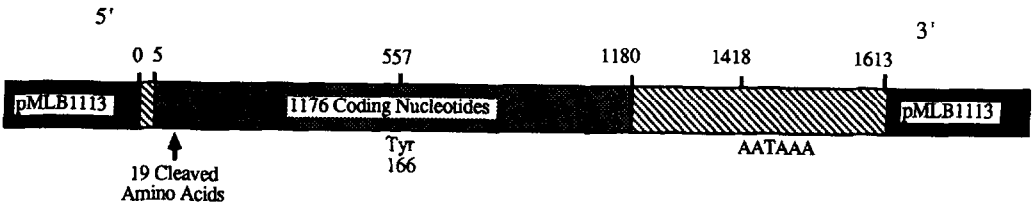

Figure 1. A physical map of the human class III ADH (ADH5) cDNA, ADH 14.1, is depicted.

strongly hybridizing clones, designated ADH5-14.1 and ADH5-30.1, were used for further analysis. The lengths of the two ADH5 cDNA inserts were approximately equal and that of ADH5-14.1 was precisely determined to be 1613 bp (Fig. 1), a size adequate to contain a full-length cDNA for the 40,000 Da class III ADH subunit.

To rule out the possibility that these clones were class I ADH cDNAs, they, as well as a full length cDNA clone for class I (β) ADH (from Dr. A. Yoshida, City of Hope, Duarte CA), were hybridized first to the class I ADH cDNA and then to the class III-specific oligonucleotides A and B. The class III ADH oligonucleotides gave strong hybridization signals with ADH5-14.1 and ADH5-30.1 but a weak signal with the class I ADH cDNA. Clones ADH5-14.1 and ADH5-30.1 revealed common internal restriction sites with *EcoR* I, *Pst* I and *Rsa* I. ADH5-14.1 was selected for initial sequencing analyses and ADH5-30.1 was used for confirmation of some sequences.

Fig. 1 depicts the overall structure of ADH5-14.1 and Fig. 2 gives the complete sequence including 433 3' flanking nucleotides and either 1122 or 1176 coding nucleotides. The 3' - nontranslated sequence contains two potential polyadenylation signals, AATAAA, 377 nucleotides downstream from the termination codon, CATAAA, 412 nucleotides downstream. However, neither is closely followed by a polyA tail. The sequences around the ATGs at positions 5 and 59, are both consistent with the consensus eukaryotic initiation sites however the ATG at position 5 is more likely to be utilized because it is further upstream (21). An open reading frame of 1122 bp extends from the ATG at position 59, encoding a protein of 374 residues. The methionine at the amino terminus would be removed making Ala the initial residue in the mature chain of 373 amino acids (10). However, the upstream ATG at position 5 initiates an open reading frame which includes 19 N-terminal amino acids which are not seen in the mature protein (10). Except for Tyr 166 of the mature chain, reported as Asp (10), the cDNA sequence and the published protein sequence are otherwise compatible.

Expression of Class III ADH in human tissues - In total RNA from liver, spleen, and cell lines mRNAs hybridizing to ADH-14.1 ranged from 1.7 kb to 5.6 kb (Fig. 3). Hybridizing bands of approximately the same sizes were detected in poly (A)+ RNA from liver. The predominant mRNA detected in all tissues was 1.7 kb, slightly smaller than 18S rRNA. The cloned class III ADH cDNAs (ADH5-14.1 and ADH5-30.1) are therefore full-length or nearly full-length. Two larger bands, 3.5 and 5.6 kb in size, generated weaker hybridization signals. Class I and II ADH probes detected different mRNA sizes in a different tissue distribution (data not shown). Hybridization with a class II ADH-specific 24-mer (5'ATTCCACTTTATGCACCTCTATGT3') revealed a band of 1.6 and 2.4 kb in human liver, with possibly some hybridization in spleen and

```
                                                    Met Gly Ala Ala Thr Pro Val
                                                GGGC ATG GGC GCG GCC ACC CCG GAT   25
Asp Ser Pro Pro Arg Arg Pro Glu Ser Val Asn Met Ala Asn Glu Val Ile Lys Cys Lys Ala Ala
GTC AGC CCC CCG CGC CGA CCA GAA TCC GTG AAC ATG GCG AAC GAG GTT ATC AAG TGC AAG GCT GCA   91

Val Ala Trp Glu Ala Gly Lys Pro Leu Ser Ile Glu Glu Ile Glu Val Ala Pro Pro Lys Ala His
GTT GCT TGG GAG GCT GGA AAG CCT CTC TCC ATA GAG GAG ATA GAG GTG GCA CCC CCA AAG GCT CAT  157

Glu Val Arg Ile Lys Ile Ile Ala Thr Ala Val Cys His Thr Asp Ala Tyr Thr Leu Ser Gly Ala
GAA GTT CGA ATC AAG ATC ATT GCC ACT GCG GTT TGC CAC ACC GAT GCC TAT ACC CTG AGT GGA GCT  223

Asp Pro Glu Gly Cys Phe Pro Val Ile Leu Gly His Glu Gly Ala Gly Ile Val Glu Ser Val Gly
GAT CCT GAG GGT TGT TTT CCA GTG ATC TTG GGA CAT GAA GGT GCT GGA ATT GTG GAA AGT GTT GGT  289

Glu Gly Val Thr Lys Leu Lys Ala Gly Asp Thr Val Ile Pro Leu Tyr Ile Pro Gln Cys Gly Glu
GAG GGA GTT ACT AAG CTG AAG GCG GGT GAC ACT GTC ATC CCA CTT TAC ATC CCA CAG TGT GGA GAA  355

Cys Lys Phe Cys Leu Asn Pro Lys Thr Asn Leu Cys Gln Lys Ile Arg Val Thr Gln Gly Lys Gly
TGC AAA TTT TGT CTA AAT CCT AAA ACT AAC CTT TGC CAG AAG ATA AGA GTC ACT CAA GGG AAA GGA  401

Leu Met Pro Asp Gly Thr Ser Arg Phe Thr Cys Lys Gly Lys Thr Ile Leu His Tyr Met Gly Thr
TTA ATG CCA GAT GGT ACC AGC AGA TTT ACT TGC AAA GGA AAG ACA ATT TTG CAT TAC ATG GGA ACC  487

Ser Thr Phe Ser Glu Tyr Thr Val Val Ala Asp Ile Ser Val Ala Lys Ile Asp Pro Leu Ala Pro
AGC ACA TTT TCT GAA TAC ACA GTT GTG GCT GAT ATC TCT GTT GCT AAA ATA GAT CCT TTA GCA CCT  553

Leu Tyr Lys Val Cys Leu Leu Gly Cys Gly Ile Ser Thr Gly Tyr Gly Ala Ala Val Asn Thr Ala
TTG TAT AAA GTC TGC CTT CTA GGT TGT GGC ATT TCA ACC GGT TAT GGT GCT GCT GTG AAC ACT GCC  619

Lys Leu Glu Pro Gly Ser Val Cys Ala Val Phe Gly Leu Gly Gly Val Gly Leu Ala Val Ile Met
AAG TTG GAG CCT GGC TCT GTT TGT GCC GTC TTT GGT CTG GGA GGA GTC GGA TTG GCA GTT ATC ATG  685

Gly Cys Lys Val Ala Gly Ala Ser Arg Ile Ile Gly Val Asp Ile Asn Lys Asp Lys Phe Ala Arg
GGC TGT AAA GTG GCT GGT GCT TCC CGG ATC ATT GGT GTG GAC ATC AAT AAA GAT AAA TTT GCA AGG  751

Ala Lys Glu Phe Gly Ala Thr Glu Cys Ile Asn Pro Gln Asp Phe Ser Lys Pro Ile Gln Glu Val
GCC AAA GAG TTT GGA GCC ACT GAA TGT ATT AAC CCT CAG GAT TTA AGT AAA CCC ATC CAG GAA GTG  817

Leu Ile Glu Met Thr Asp Gly Gly Val Asp Tyr Ser Phe Glu Cys Ile Gly Asn Val Lys Val Met
CTC ATT GAG ATG ACC GAT GGA GGA GTG GAC TAT TCC TTT GAA TGT ATT GGT AAT GTG AAG GTC ATG  883

Arg Ala Ala Leu Glu Ala Cys His Lys Gly Trp Gly Val Ser Val Val Val Gly Val Ala Ala Ser
AGA GCA GCA CTT GAG GCA TGT CAC AAG GGC TGG GGC GTC AGC GTC GTG GTT GGA GTA GCT GCT TCA  949

Gly Glu Glu Ile Ala Thr Arg Pro Phe Gln Leu Val Thr Gly Arg Thr Trp Lys Gly Thr Ala Phe
GGT GAA GAA ATT GCC ACT CGT CCA TTC CAG CTG GTA ACA GGT CGC ACA TGG AAA GGC ACT GCC TTT 1015

Gly Gly Trp Lys Ser Val Glu Ser Val Pro Lys Leu Val Ser Glu Tyr Met Ser Lys Lys Ile Lys
GGA GGA TGG AAG AGT GTA GAA AGT GTC CCA AAG TTG GTG TCT GAA TAT ATG TCC AAA AAG ATA AAA 1081

Val Asp Glu Phe Val Thr His Asn Leu Ser Phe Asp Glu Ile Asn Lys Ala Phe Glu Leu Met His
GTT GAT GAA TTT GTG ACT CAC AAT CTG TCT TTT GAT GAA ATC AAC AAA GCC TTT GAA CTG ATG CAT 1147

Ser Gly Lys Ser Ile Arg Thr Val Val Lys Ile Term
TCT GGA AAG AGC ATT CGA ACT GTT GTA AAG ATT TAA  1183

TTCAAAAGAG AAAAATAATG TCCATCCTGT CGTGATGTGA TAGGAGCAGC TTAACAGGCA GGGAGAAGCG CCTCCAACCT 1263
CACAGCCTCG TAGAGCTTCA CAGCTACTCC AGAAAATAGG GTTATGTGTG TCATTCATGA ATCTCTATAA TCAAGGACAA 1343
GGATAATTCA GTCATGAACC TGTTTTCTGG ATGCTCCTCC ACATAAATAA TTGCTAGTTA TAAGGATATT TAACATAATA 1423
AAAGTAATTC TACATTGTGT GAATTGTCTT GTTTATGCTG TCATCATTGT CACGGTTTGT CTGCCCATTA TCTTCATTCT 1503
GCAAGGGAAA GGGAAAGGAA GCAGGGCAGT GGTGGGTGTC TGAAACCTCA GAAACATAAC GTTGAACTTT TAAGGGTCTC 1583
AGTCCCCGTT GATTAAAGAA CAGATCCCCG 1613
```

Figure 2. The complete nucleotide and predicted amino acid sequences of human class III ADH (ADH5). Tyr-166 of the mature chain, reported on the basis of amino acid sequencing to be Asp, is underlined. The 19 N-terminal amino acids which are probably coded but cleaved are italicized. Two potential polyadenylation signals are underlined.

in the neuroblastoma line, SK-N-MC [data not shown]. For class I ADH, a β cDNA probe (obtained from A. Yoshida) detected abundant message in human and hamster livers and generated a weaker signal with human spleen RNA [as previously described (22) and data not shown].

Mapping of class III-like ADH to mouse chromosome 3 - Hybridization of the ADH5 probe to *Hind* III-digested genomic DNA revealed hamster DNA fragments of 3.3, 1.95, 1.8 and 1.15 kb and mouse DNA fragments of 3.0, 1.65 and 0.6 kb. Because it was most readily scored, the presence of the mouse 3.0 kb fragment was followed in a set of mouse-hamster cell lines which have been characterized for the presence of mouse chromosomes (23). The 1.65 and 0.6 kb fragments showed identical distributions. As can be seen in Table I, there was no discordance

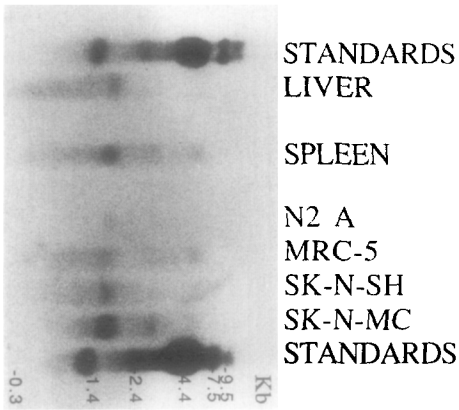

Figure 3. RNA "Northern" blot hybridization, demonstrating the occurrence of the ADH5 mRNA in human liver, spleen and various human cell lines, as indicated. Total RNA (10 μg) was electrophoresed, transferred to a nylon membrane and hybridized to the ADH5 14.1 cDNA probe labeled by the random primer method.

between chromosome 3 and the 3.0 kb class III-like ADH fragment. All other chromosomes were more than 10% discordant.

Mapping of class III ADH to human chromosome 4- Hybridization of the ADH5 cDNA probe to hamster, mouse and human genomic DNAs digested with *Hind* III revealed a 9 kb fragment distinct for the human. The fragment's presence was followed in mouse-human and hamster-human cell lines characterized enzymatically and karyotypically for the presence of human chromosomes (24). Hybrid lines with human chromosome 4 were 5.7% discordant for the 9 kb fragment and all other chromosomes were more than 10% discordant. Also, hybrid lines expressing the human form of PEPS, a chromosome 4 enzyme marker, were 5.3% discordant.

DISCUSSION

The unusualness of class III ADH was emphasized on the basis of its enzymatic properties, ubiquitous distribution (including expression in brain and testes) and closer protein sequence

Table I
Distribution of a Mouse Class III-like ADH Fragment Across Mouse/Hamster Hybrid Cell Lines

	+/+	+/-*	-/+	-/-	Total	Disc**		+/+	+/-	-/+	-/-	Total	Disc
Chr 1	4	2	0	6	12	0.167	Chr 11	0	5	0	5	10	0.500
Chr 2	5	2	2	4	13	0.308	Chr 12	3	3	2	2	10	0.500
Chr 3	6	0	0	4	10	0.000	Chr 13	2	4	2	3	11	0.545
Chr 4	2	5	1	5	13	0.462	Chr 14	0	7	1	5	13	0.615
Chr 5	1	5	1	4	11	0.545	Chr 15	5	0	2	2	9	0.222
Chr 6	3	4	2	3	12	0.500	Chr 16	1	5	2	3	11	0.636
Chr 7	4	3	1	3	11	0.364	Chr 17	6	1	1	3	11	0.182
Chr 8	3	4	1	4	12	0.417	Chr 18	2	4	1	5	12	0.417
Chr 9	2	5	1	4	12	0.500	Chr 19	3	4	1	4	12	0.417
Chr 10	1	6	0	5	12	0.500	Chr X	6	1	1	5	13	0.154

* +/- The mouse class III-like ADH is present yet the chromosome is absent.
** Disc = discordance.

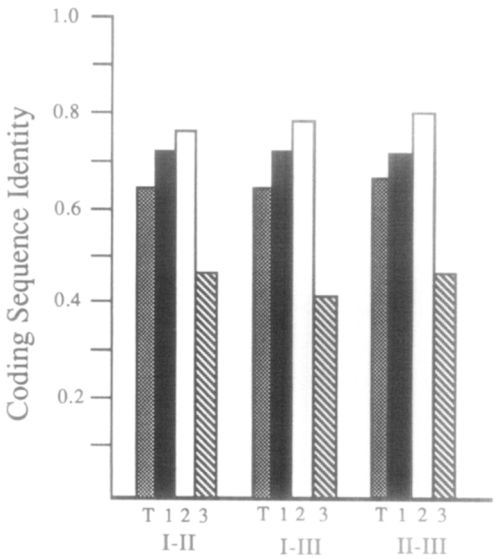

Figure 4. The extent of interclass similarity among the human ADHs. Nucleotide sequence identities are given as totals(T) and for the first, second and third bases of codons.

identity to the plant ADHs (10); however in protein sequence identity the three ADH classes are equidistant (10). DNA sequence comparisons leave open the question of whether class III ADH is in some features more functionally conserved but yield the definitive conclusion that class III ADH is closer to the other mammalian ADHs than to the plant ADHs. Coding DNA sequence identity of class III ADH was 67% to class II ADH, 65% to class I ADH (γ), and 58% to the maize ADH1-F, while the identity between class I and II ADH is 65% (Fig 4). The same relative order of similarity is observed at the first and second bases of codons where functional constraints are highest and at the third base of the codon where synonymous substitutions generally occur (Fig. 4). Therefore, the three ADH classes are approximately equidistant and the existence of greater functional constraints for a particular ADH class is not obvious at the level of overall sequence identity.

The origin of the observed multiple class III ADH mRNA transcripts is unknown, however they do not represent cross-hybridization to the larger class I and II RNAs, which are of different sizes than the larger class III ADH transcripts. We were able to detect two poly (A) addition signals in the 3' flanking region, but did not detect the site of poly (A) addition. The presence of larger unprocessed RNA transcripts ranging up to 5.6 kb in size raises the possibility that additional signal sequences are present downstream. We have not ruled out the possibility of alternative splicing, the existence of incompletely processed transcript or a polycistronic mRNA. Ikuta *et al.* recently reported the isolation of a fused human α and β ADH cDNA that may have been generated from a natural 4.3 kb class I ADH mRNA fragment (25).

Sequencing of the cDNA encoding class III ADH revealed two differences between the deduced amino acid sequence and the published protein sequence. The first is the probable existence of a 19 amino acid N-terminal sequence which would subsequently be cleaved. Second, where Asp-166 was found (10), the cDNA sequence specifies Tyr. This difference, due to a single base substitution (GAT to TAT) could be a copy error by reverse transcriptase during cDNA

preparation. A second possibility is an error in the sequencing of peptides or DNA. However, the published amino acid sequence of class III ADH at this position was from several overlapping peptides. For our DNA sequencing, sequences were confirmed on opposite strands and with a second, possibly independent, clone (ADH5-30.1). A third possibility is polymorphism.

Finally, the cloned cDNA and sequenced protein may represent different class III ADH loci. Three class I ADH loci are known, and sequence differences between the class II ADH protein and cDNA could be explained by the existence of multiple loci (9). The substitution of Tyr for Asp-166 in class III ADH would result in a detectable alteration in electrophoretic mobility and isoelectric point. The well-known electrophoretic heterogeneity of class III ADH is therefore intriguing; three bands are detected by starch gel electrophoresis (5) and by isoelectric focusing; (χ1, χ2 and χ3) having isoelectric points of 5.8, 6.1 and 6.4 (26). In this regard, the class III ADH sequenced by Kaiser *et al*. consisted largely of χ1. However, Adinolfi *et al*. showed that χ1, χ2 and χ3 co-vary and suggested that χ2 and χ3 are derived by modification of a single translation product.(6). Chemically modified forms of class I ADH have been observed (27-28).

Close clustering of class III ADH with other ADHs on human chromosome 4 and mouse chromosome 3 is strongly suggested by the conservation of synteny of these genes between the two species. The three human class I ADH loci were assigned by *in situ* hybridization to 4q21-23 (29). By analogy to other clustered genes of related sequence and function, it can be postulated that there will be important implications in the areas of gene regulation, mutation and evolution.

REFERENCES

1. Vallee, B.L. and Bazzone, T.J (1984) in Isozymes: Current Topics in Biological and Medical Research (Rattazzi, H.C., Scandalios, J.G. and Whitt, G.S., eds) Vol. 8, pp 219-264, A.R.Liss, NY.
2. Julia, P., Farres, J. and Pares, X. (1987) Eur. J. Biochem. 162, 179-189.
3. Bosron, W. F., Li, T. K., Dafeldecker, W. P., and Vallee, B. L.(1979) Biochemistry 18, 1101-1105.
4. Bosron, W. F., Li, T. K., Lange, L. G., Dafeldecker, W. P. and Vallee, B. L. (1977) Biochem. Biophys. Res. Commun. 71, 85-91.
5. Pares, X. and Vallee, B. L. (1981) Biochem. Biophys. Res. Commun. 98, 122-130.
6. Adinolfi, A., Adinolfi, M. and Hopkinson, D. A. (1984) Ann. Hum. Genet. 48, 1-10.
7. Rathnagiri, P., Linnoila, M., O'Neill, J.B. and Goldman, D. (1989) Brain Res. 481, 131-141.
8. Ikuta, T., Fujiyoshi, T., Klurachi, K. and Yoshida, A. (1985) Proc. Natl. Acad. Sci. USA 82, 2703-2707.
9. Hoog, J-O., Von Bahr Lindstrom, H., Heder, L-O., Holmquist, B., Larsson,K., Hempel, J., Vallee. B.L. and Jornvall, H. (1987) Biochemistry 26, 1926-1932.
10. Kaiser, R., Holmquist, B., Hempel, J., Vallee, B.L. and Jornvall, H. (1988) Biochemistry 27, 1132-1140.
11. Wagner, F.W., Pares, X., Holmquist, B. and Vallee, B.L.(1984) Biochemistry 23, 2193-2199.
12. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory.
13. Hanes, B.D. and Higgins, S.J.(eds) (1985) Nucleic acid hybridization: A practical approach. IRL Press.
14. Zagursky, R. and Berman, M.L. (1984) Gene 27:183-191.
15. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
16. Messing, J. and Vieira, J. (1982) Gene 19, 269-276.
17. Zagursky, R., Baumeister, K., Lamak, N. and Berman, M. (1985) Gene Anal. Techn. 2, 89-94.

18. Cathala, G., Savouret, J.F., Mendez, B., West, B.L., Karin, M., Martial, J.A. and Baxter, J.D. (1983) DNA 2, 329-335.
19. Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408-1412.
20. Chen-Kiang, S., Wolgemuth, D.J., Hsu, M.T. and Darnell, J.E. (1982) Cell 28, 575-584.
21. Kozak, M. (1984) Nucleic Acids Res. 12, 857-872.
22. Ikuta, T. and Yoshida, A. (1986) Biochem. Biophys. Res. Comm. 140, 1020-1027.
23. Hoggas, M.D., Halden, N.F., Buckler, C.E. and Kozak, C.A. (1988) J. Virology 62, 1055-1056.
24. O'Brien, S.J., Simonson, J.M. and Eichelberger, M.A. (1982) in Techniques in Somatic Cell Genetics. (Shay, J.W., ed), pp. 513-524, Plenum Press, NY.
25. Ikuta, T., Szeto, S. and Yoshida, A. (1986) Proc. Natl. Acad. Sci. USA 83, 634-638.
26. Valkonen, K.H. and Goldman, D. (1986) Protides of the Biological Fluids 34, 785-789.
27. Pietruszko, R. and Theorell, H. (1969) Arch. Biochem. Biophys. 131, 288-298.
28. Jornvall, H. (1973) Biochem. Biophys. Res. Commun. 53, 1096-1101.
29. Tsukahara, M. and Yoshida, A. (1989) Genomics 46, 218-220.