Am. J. Hum. Genet. 41:1025–1034, 1987

Human Placental and Intestinal Alkaline Phosphatase Genes Map to 2q34-q37

CONSTANCE A. GRIFFIN,*[,1] MOYRA SMITH,§ PAULA S. HENTHORN,†[,‡] HARRY HARRIS,‡ MITCHELL J. WEISS,‡ MICHAEL RADUCHA,‡ AND BEVERLY S. EMANUEL*

*Departments of Pediatrics and Genetics and †Division of Biochemical Development, Children's Hospital of Philadelphia; ‡Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia; and §Department of Pediatrics, University of California, Irvine

SUMMARY

The alkaline phosphatases comprise a multigene enzyme family that hydolyze phosphate esters and are widely distributed in nature. Three main classes have been isolated from humans, the placental, intestinal, and liver/bone/kidney forms. We have mapped the placental and intestinal alkaline phosphatase genes to 2q34-q37 by using chromosomal in situ hybridization and a somatic-cell hybrid panel.

INTRODUCTION

The alkaline phosphatases (ALPs; orthophosphoric-monoester phosphohydrolase [alkaline optimum], E.C.3.1.3.1) are membrane-bound glycoproteins that hydrolyze a variety of phosphate esters having high pH optima (McComb et al. 1979). In humans, a number of different forms of these enzymes are found in different tissues. Inhibition, thermostability, immunologic, and electrophoretic studies have been used to categorize ALPs isolated from various tissues into three main classes—placental, intestinal, and liver/bone/kidney (L/B/K) (Mulivor et al. 1978*a;* McKenna et al. 1979; Seargeant and Stinson 1979; Harris 1982)—that appear to be determined by at least three gene loci. It is probable that there are further ALP loci. For instance, intestinal ALPs in the fetus and adult show electrophoretic and immunologic differences that suggest that these

Received January 26, 1987.

Address for correspondence and reprints: Beverly S. Emanuel, Children's Hospital of Philadelphia, Departments of Pediatrics and Genetics, Philadelphia, PA 19104.

1. Present address: The Johns Hopkins Oncology Center, Baltimore, MD 21205.

protein moieties may be encoded by separate loci (Mulivor et al. 1978*b;* Vockley et al. 1984*a,* 1984*b;* Mueller et al. 1985). In addition, an unusual placental-like ALP is detected in small amounts in testis and thymus and may be the product of yet another locus (Chang et al. 1980; Goldstein et al. 1982; Millan and Stigbrand 1983).

It is thought that the ALPs are members of a multigene enzyme family that arose by successive duplications from a common ancestral gene (Harris 1982). Immunologic evidence suggests that the placental and intestinal ALPs are more closely related to each other than either is to L/B/K ALP. The isolation and sequencing of various ALP cDNAs (Kam et al. 1985; Henthorn et al. 1986, 1987; Millan 1986; Weiss et al. 1986) confirms the existence of at least three related ALP gene loci. The amino acid sequences deduced from placental and intestinal ALP cDNAs are 86% homologous. In contrast, deduced amino acid sequences of placental and intestinal ALPs show 52% and 55% homology, respectively, to the L/B/K ALP–deduced amino acid sequence (Weiss et al. 1986; Henthorn et al. 1987). In light of these relationships between members of the ALP gene family, the chromosomal locations of the individual family members are of particular interest. We report here the use of cDNA probes to map the human placental and human intestinal ALP loci to the distal end of the long arm of chromosome 2.

MATERIAL AND METHODS

Probes

The placental ALP cDNA clone used as probe (*ALPP*) consists of a 1.4-kb *Sst*I fragment isolated from a full-length type 3 placental ALP cDNA clone (Henthorn et al. 1986) inserted into the plasmid vector pGEMI (Promega Biotec, Madison, WI). This insert consists of approximately half protein-coding and half 3′-untranslated sequences.

The intestinal ALP cDNA used as probe (*ALPI*) is the cDNA clone D98#7 (described in Henthorn et al. 1987) also constructed in the plasmid vector pGEMI. This 2.45-kb cDNA insert was shown to encode intestinal ALP by the identity of both partial sequences (~1,000 bases) and restriction-enzyme mapping data to a full-length intestinal ALP cDNA that was completely sequenced (Henthorn et al. 1987).

In Situ Hybridization

Plasmids containing the *ALPP* and *ALPI* inserts were ^{3}H-labeled by means of nick-translation to a specific activity of 2×10^{7} cpm/μg. Each probe was hybridized to human metaphase chromosome preparations made from the short-term culture of peripheral lymphocytes of normal males. Hybridization was essentially according to the method of Harper et al. (1981) at 37 C for 15–18 h in 50% formamide, 2 × SSC (1 × SSC = 0.15 M sodium chloride, 0.15 M sodium citrate), 10% dextran sulfate with 100 μg sonicated salmon-sperm DNA/ml as carrier. Washes were performed at 39 C in 50% formamide, 2 × SSC for 9 min, then in 2 × SSC for 12 min, followed by dehydration in ethanol. Autoradio-

graphy was performed using NTB-2 Kodak emulsion for 15–21 days before development. Chromosome banding was achieved using borate buffer and Wright-Giemsa stain (Cannizzaro and Emanuel 1984). Only well-spread, banded metaphases were used for identification of the location of grains localized on chromosomes.

Hybrid Cell Lines Used

Somatic-cell hybrids Sif 4D2A, Sif 4D2F, Fst II, FU5 V2, and FU5 V7A were derived from fusions between the hypoxanthine guanine phosphoribosyl transferase (HGPRT)–deficient rat hepatoma-cell lines—i.e., the Faza and FU5 lines—and primary human fibroblast cultures from three different individuals. Hybrids HHW 509, HHW 455, KO1 (HHW 416), KO8 (HHW 661), and B7-10 were derived from fusion of temperature-sensitive Chinese hamster ovary cells (CHO) mutant in tRNA synthetase genes (Carlock and Wasmuth 1985). The hybrid CF84/11 was derived from the fusion of the thymidine kinase (TK)–deficient cell line GM34 with normal human fibroblasts (T. Mohandas, personal communication). Hybrid cells produced by fusion with the HGPRT- or TK-deficient rodent cell lines were grown in hypoxanthine-aminopterin-thymidine (HAT) medium (Littlefield 1966). Hybrids derived from fusion with temperature-sensitive CHO cell lines were grown at 39 C.

The human chromosomal content of hybrid clones was determined using several methods. Human chromosomes were distinguished from those of rodents by using Giemsa-11 staining (Friend et al. 1976) and trypsin-Giemsa banding (Seabright 1971). Analyses of marker enzymes and DNA probes with known assignments to specific human chromosomes were also carried out. Cell extracts were subjected to starch-gel or cellogel electrophoresis under conditions that separate human, rodent, and hybrid enzyme forms (Harris and Hopkinson 1976). Individual hybrid clones were harvested at the same passage number used for chromosomes, enzyme analyses, and analyses with DNA probes.

Southern Blot Analysis

Genomic DNA extracted from human, rodent, and somatic-cell hybrids was digested to completion with restriction endonucleases *Bam*HI or *Hin*dIII. Ten micrograms parental cell DNA/lane or 20 μg hybrid cell DNA/lane was loaded onto 0.8% agarose gels. The DNA fragments were separated by means of electrophoresis and then transferred onto nitrocellulose filters (Southern 1975). ALP gene inserts were separated from their plasmid vectors by means of restriction-endonuclease digestion, agarose electrophoresis, and electroelution. ALP inserts were labeled with ^{32}P dATP by using the random primer method (Feinberg and Vogelstein 1983). Prehybridization and hybridization of filters were carried out in the presence of 50% deionized formamide, 6 × SSC, 5 × Denhardt's solution (50 × Denhardt's = 0.5 g Ficoll, 0.5 g polyvinylpyrrolidone, 0.5 g bovine serum albumin in 100 ml water), 0.1% sodium dodecyl sulfate (SDS), 200 μg salmon-sperm DNA/ml, and 10 μg polyA RNA/ml at 42 C (Maniatis et al. 1982). After hybridization with labeled probe, filters were

washed first at room temperature and then twice at 60 C for 20 min in 1 × SSC and 0.1% SDS. Filters were autoradiographed for 48–96 h at −70 C with the use of Kodak XAR film and DuPont intensifying screens.

RESULTS AND DISCUSSION

ALPP and *ALPI* probes were hybridized to DNAs from a somatic-cell hybrid panel. In human genomic DNA, both probes hybridized to *Bam*HI fragments of sizes 10.0, 7.7, and 3.0 kb (figs. 1*A*, 1*B*) and to *Hin*dIII fragments of 10.1 and 4.1 kb (fig. 1*C*). This degree of cross-hybridization is not surprising in light of the high degree of homology between the two probes. However, the 7.7-kb *Bam*HI fragment was the most prominent when the *ALPI* probe was used, whereas the 10.0-kb *Bam*HI fragment was most prominent when the *ALPP* probe was used (see fig. 1), allowing us to discriminate between the two probes. Under the medium-stringency conditions that we used, no fragments of corresponding sizes were present in DNA from the rodent cell lines. Therefore, hybrids that contained 10.0-, 7.7-, and 3-kb *Bam*HI fragments and 10.1- and 4.1-kb *Hin*dIII fragments were scored as positive for the genes that code for human placental and intestinal alkaline phosphatase. Table 1 shows that the only hybrid that was positive for human alkaline phosphatase bands was hybrid Sif 4D2F. The only chromosome present in Sif 4D2F and absent from the other hybrid clones is human chromosome 2.

In situ hybridization with tritium-labeled probes containing the *ALPP* and *ALPI* inserts showed specific hybridization to chromosome 2 in the region q34-q37. Two separate experiments were performed with each probe, and a total of 100 metaphases were scored for each probe. A total of 178 chromosomally localized grains were evaluated for the *ALPP* probe, and 56 (31%) of these were localized to 2q34-q37. Localization of the *ALPI* probe was similar, with 84 (44%) of 191 chromosomally localized grains localized to 2q34-2q37. Figure 2*A* shows the distribution of silver grains over the entire genome for the *ALPP* probe, and figure 2*B* does so for the *ALPI* probe. With neither probe is there any significant hybridization to any other site in the genome.

Our data confirm the report of Kam et al. (1985) that placental alkaline phosphatase sequences are present on chromosome 2, and it now more specifically defines the location of these sequences as 2q34-q37. We did not detect hybridization to chromosome 17 with our *ALPP* probe in either in situ hybridization or in DNA from the hybrid that contained chromosome 17 (CF84/11), and we thus do not confirm their report of sequences homologous to placental alkaline phosphatase on chromosome 17.

In addition, our data show that the intestinal alkaline phosphatase gene also

FIG. 1.—Southern blots of human, hamster, and hybrid cell line DNAs hybridized to probes for alkaline phosphatase genes. *A*, Presence of the 10.0-kb band detected by the *ALPP* probe in human cell lines GM4881 and GM6017 and in hybid line Sif 4D2F DNA digested with *Bam*HI. The 3.0- and 7.7-kb bands are barely visible in the human lines with this probe. *B*, Presence of the 10.0-, 7.7-, and 3.0-kb bands detected by the *ALPI* probe in the same cell lines as above digested with *Bam*HI. *C*, The 10.1- and 4.1-kb bands detected by the *ALPI* probe in hybrid and parental cell line DNAs digested with *Hin*dIII.

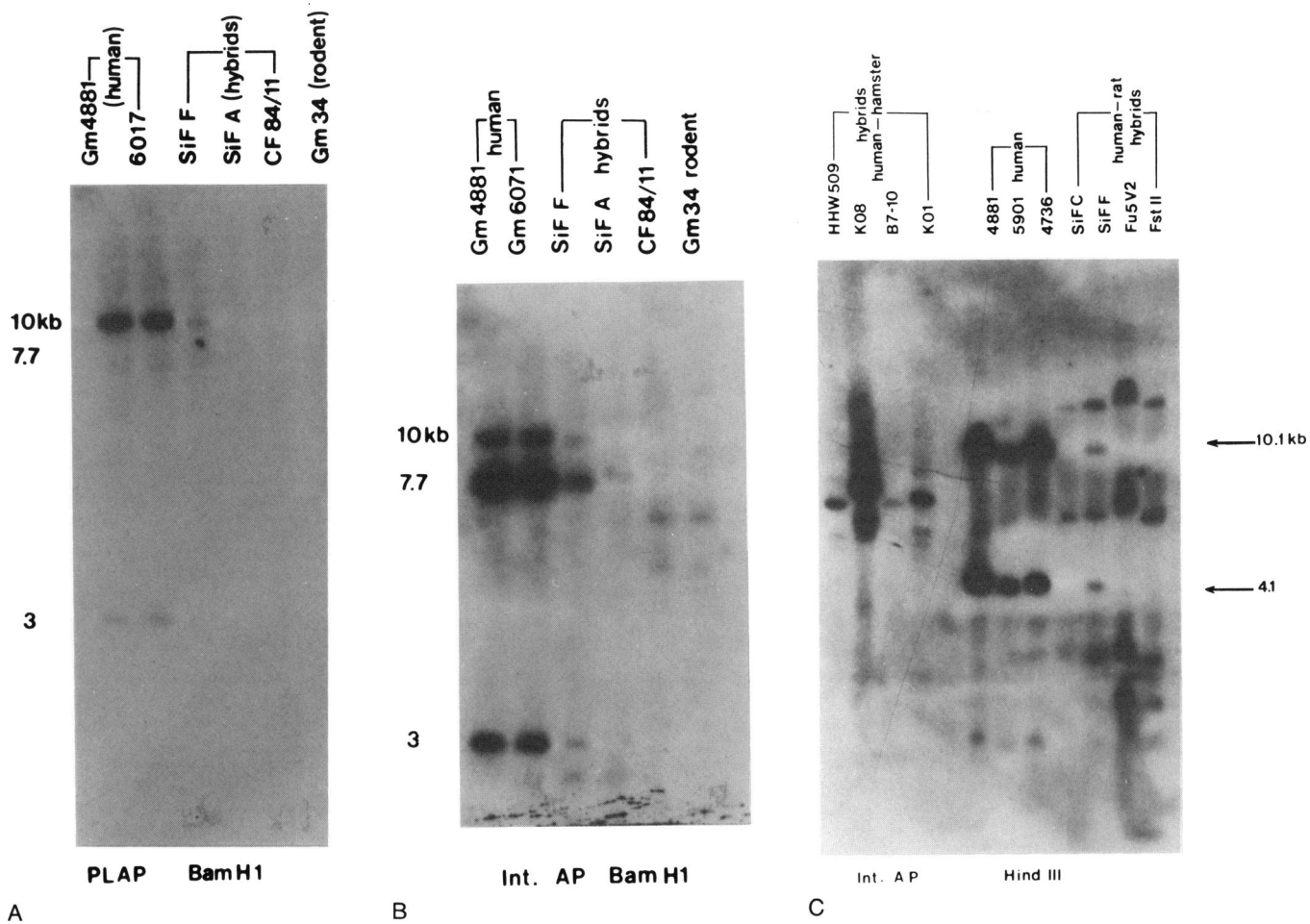
Gm4881 (human)
6017
SiF F
SiF A (hybrids)
CF 84/11
Gm 34 (rodent)
10kb
7.7
3
PLAP
BamH1
A
Gm 4881 human
Gm 6071
SiF F
SiF A hybrids
CF 84/11
Gm34 rodent
10kb
7.7
3
Int. AP
BamH1
B
HHW 509
K08
B7-10
K01
hybrids human—hamster
4881
5901
4736
human
SiF C
SiF F
Fu5 V2
Fst II
human—rat hybrids
10.1 kb
4.1
Int. AP
Hind III
C

TABLE 1

SEGREGATION OF *ALPP* AND *ALPI* LOCI IN SOMATIC-CELL HYBRIDS

HYBRID CLONE	IAP	PLAP	CHROMOSOME																						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Sif 4D2A	−	−				+						+	+			+									+
Sif 4D2C	−	−			+[b]	+	+	+	+		+		+	+		+	+	+		+			+	+	+
Sif 4D2F[a]	+	+		+		+						+	+	+			+			+	+	+	+		+
Fst II	−	−	+			+		+		+		+					+			+	+				+
FU5 V2	−	−			+	+	+	+	+	+	+	+	+	+		+	+	+			+	+				+
FU5 V7A	−	−	+			+	+	+				+	+	+	+	+	+				+	+	+		+
HHW 509	−	−					+			+															
HHW 455	−	−				+[b]																			
KO1 (HHW 416)	−	−				+																			
KO8 (HHW 661)	−	−				+[c]								+[c]											
B7-10	−	−			+	+	+	+			+			+		+				+				+	
CF84/11	−	−																	+		+				

NOTE.—Hybrid cell lines were examined cytogenetically and/or with enzyme or molecular probes for the presence of each human chromosome. A plus sign (+) indicates presence of the chromosome.

[a] The only hybrid containing chromosome 2, and the only hybrid cell line to which the *ALPP* and *ALPI* probes hybridized.

[b] Only the p arm of the chromosome present.

[c] Derivative chromosome 4qter-4q23::12p13-12pter present.

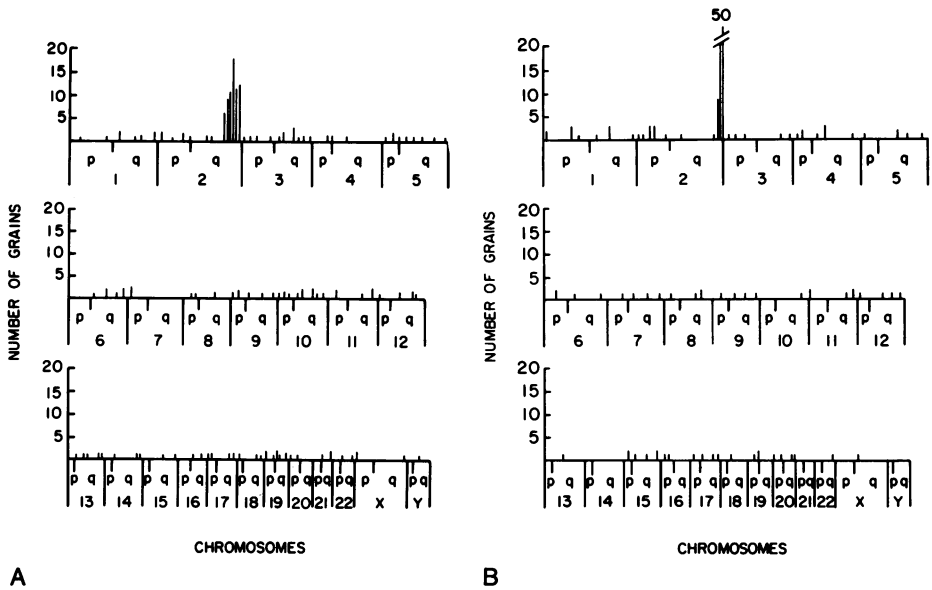

FIG. 2—Grain distribution from in situ hybridizations. The histograms show the grain distribution in human metaphase spreads when the alkaline phosphatase genes were used as probes. The abscissa represents the chromosomes in their relative size proportions, and the ordinate shows the number of silver grains. *A, ALPP* as the probe; *B, ALPI* as the probe.

maps to the distal end of chromosome 2, and this also appears to be the only location of these sequences in the genome. Both the *ALPI* and *ALPP* probes detect bands of identical sizes in human genomic DNA, and cross-hybridization to both *ALPI* and *ALPP* sequences probably occurred in our in situ hybridization and somatic-cell hybrid screening. However, both the absence of any secondary site of hybridization elsewhere in the genome and the intensity of the hybridization in chromosomal in situ localization experiments suggest that the *ALPI* and *ALPP* loci can be subregionally mapped within several chromosomal bands on the long arm of chromosome 2 and that they are possibly quite close to each other. Probes containing only nonhomologous flanking sequences will be required to (1) sublocalize the *ALPI* and *ALPP* loci within the q34-q37 region of chromosome 2 and (2) determine the distance between the two loci by means of chromosomal walking or long-range restriction mapping with pulse-field gel electrophoresis.

Immunologic and sequence data suggest that the separation of the placental and intestinal alkaline phosphatase genes was a more recent event, in evolutionary terms, than the separations of either from the gene coding for the L/B/K form of alkaline phosphatase (Harris 1982; Henthorn et al. 1987). This proposed evolutionary history is consistent with our mapping data, showing the location of both loci on the distal end of the long arm of chromosome 2 whereas the L/B/K gene has been shown to map to chromosome 1 (Swallow et al. 1986; M. Smith, C. A. Griffin, B. S. Emanuel, P. S. Henthorn, M. J. Weiss, and H.

Harris, unpublished data), and thus there may be two separate multigene clusters containing the different alkaline phosphatase genes (a distribution that is similar to that of the alpha- and beta-globin genes [Deisseroth et al. 1977, 1978; Jeffreys et al. 1979])—or more than two (as is the case for the collagen genes [summarized in Griffin et al. 1987]).

The functional role of the alkaline phosphatase enzymes has not yet been defined, and diseases due specifically to abnormal placental or intestinal gene loci have yet to be identified. "Ectopic" expression of placental or placental-like ALP by malignant tumors has been described (Fishman et al. 1968; Inglis et al. 1973), and it would be of interest to know whether any of these tumors contain a consistent chromosomal abnormality involving distal 2q that might alter expression of these or related genes. Knowledge of the chromosomal locations of these and additional alkaline phosphatase genes will further our understanding of both the relationships between the members of this multienzyme gene family and their possible relationships to human disease.

ACKNOWLEDGMENTS

We thank Anita Hawkins for technical assistance and Drs. T. Mohandas, M. S. Povey, L. Carlock, and J. Wasmuth for providing certain of the hybrid cell lines used in this study. This work was supported by National Institutes of Health (NIH) grant GM 27018 and March of Dimes grant 858 (to H.H.), NIH Training Grant HD 07107 (to P.S.H.), NIH Medical Scientist Training Program grant GM 07170 (to M.J.W.), NIH Training Grant CA09485-01 (to C.A.G.), NIH grant GM 32592 (to B.S.E.), and the NIAAA Intramural Research Program (M.S.).

REFERENCES

Cannizzaro, L. A., and B. S. Emanuel. 1984. An improved method for G-banding chromosomes after in situ hybridization. Cytogenet. Cell Genet. **38**:308–309.

Carlock, L. R., and J. J. Wasmuth. 1985. Molecular approach to analyzing the human 5p deletion syndrome, cri-du-chat. Somatic Cell Mol. Genet. **11**:267–276.

Chang, C. H., D. Angellis, and W. H. Fishman. 1980. Presence of a rare D-variant heat-stable, placental type alkaline phosphatase in normal human testis. Cancer Res. **40**:1506–1510.

Deisseroth, A., A. Nienhuis, J. Lawrence, R. Gilles, P. Turner, and F. H. Ruddle. 1978. Chromosomal localization of human beta globin gene on human chromosome 11 in somatic cell hybrids. Proc. Natl. Acad. Sci. USA **75**:1456–1460.

Deisseroth, A., A. Nienhuis, P. Turner, R. Velez, W. F. Anderson, F. Ruddle, J. Lawrence, R. Creagan, and R. Kucherlapati. 1977. Localization of the human alpha globin structural gene to chromosome 16 in somatic cell hybrids by molecular hybridization assay. Cell **12**:205–218.

Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.

Fishman, W. H., N. R. Inglis, S. Green, C. L. Anstiss, N. K. Gosh, A. E. Reif, R. Rustigian, M. J. Krant, and L. L. Stolbach. 1968. Immunology and biochemistry of Regan isoenzyme of alkaline phosphatase in human cancer. Nature **219**:697–699.

Friend, K. K., B. P. Dorman, R. S. Kucherlapati, and F. H. Ruddle. 1976. Detection of interspecific translocations in mouse human hybrids by alkaline Giemsa staining. Exp. Cell Res. **99**:31–96.

Goldstein, D. J., C. Rogers, and H. Harris. 1982. A search for trace expression of

placental-like alkaline phosphatase in non-malignant human tissues: demonstration of its occurrence in lung, cervix, testis and thymus. Clin. Chem. Acta **125**:63–75.

Griffin, C. A., B. S. Emanuel, J. F. Hansen, W. B. Cavenee, and J. C. Myers. 1987. Human procollagen genes encoding basement membrane 1(IV) and 2(IV) chains map to the distal long arm of chromosome 13. Proc. Natl. Acad. Sci. USA **84**:512–516.

Harper, M. E., A. Ullrich, and G. F. Saunders. 1981. Localization of the human insulin gene to the distal end of the short arm of chromosome 11. Proc. Natl. Acad. Sci. USA **78**:4458–4460.

Harris, H., ed. 1982. Multilocus enzyme systems and the evolution of gene expression. Pp. 95–124 *in* The Harvey lectures: series 76. Academic Press, New York.

Harris, H., and D. A. Hopkinson. 1976. Handbook of enzyme electrophoreses in human genetics. North-Holland, Amsterdam.

Henthorn, P. S., B. J. Knoll, M. Raducha, K. N. Rothblum, C. Slaughter, M. Weiss, M. A. Lafferty, T. Fisher, and H. Harris. 1986. Products of two common alleles at the locus for human placental alkaline phosphatase differ by seven amino acids. Proc. Natl. Acad. Sci. USA **83**:5597–5601.

Henthorn, P. S., M. Raducha, Y. H. Edwards, M. J. Weiss, C. Slaughter, M. A. Lafferty, T. Fisher, and H. Harris. 1987. Nucleotide and amino acid sequences of human intestinal alkaline phosphatase: close homology to placental alkaline phosphatase. Proc. Natl. Acad. Sci. USA **84**:1234–1238.

Inglis, N. R., S. Kirley, L. L. Stolbach, and W. H. Fishman. 1973. Phenotypes of the Regan isoenzyme and identity between the placental D-variant and the nagao isoenzyme. Cancer Res. **33**:1657–1661.

Jeffreys, A. J., I. W. Craig, and U. Francke. 1979. Localization of the G-gamma, A-gamma, delta, and beta globin genes on the short arm of human chromosome 11. Nature **281**:606–608.

Kam, W., E. Clauser, Y. S. Kim, Y. W. Kan, and W. J. Rutter. 1985. Cloning, sequencing and chromosomal localization of human term placental alkaline phosphatase cDNA. Proc. Natl. Acad. Sci. USA **82**:8715–8719.

Littlefield, J. W. 1966. The use of drug resistance markers to study the hybridization of mouse fibroblasts. Exp. Cell Res. **41**:190–195.

McComb, R. B., G. N. Bowers, Jr., and S. Posen. 1979. Alkaline phosphatase. Plenum, New York.

McKenna, M. J., T. A. Hamilton, and H. H. Sussman. 1979. Comparison of human alkaline phosphatase isozymes: structural evidence for three protein classes. Biochem. J. **181**:67–73.

Maniatis, T., E. F. Fristch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Millan, J. L. 1986. Molecular cloning and sequence analysis of human placental alkaline phosphatase. J. Biol. Chem. **261**:3112–3115.

Millan, J. L., and T. Stigbrand. 1983. Antigenic determinants of human placental and testicular placental-like alkaline phosphatases as mapped by monoclonal antibodies. Eur. J. Biochem. **136**:1–7.

Mueller, H. D., H. Leung, and R. A. Stinson. 1985. Different genes code for alkaline phosphatases from human fetal and adult intestine. Biochem. Biophys. Res. Commun. **126**:427–433.

Mulivor, R. A., V. L. Hanning, and H. Harris. 1978*a*. Developmental change in human intestinal alkaline phosphatase. Proc. Natl. Acad. Sci. USA **75**:3909–3912.

Mulivor, R. A., L. I. Plotkin, and H. Harris. 1978*b*. Differential inhibition of the products of the human alkaline phosphatase loci. Ann. Hum. Genet. **42**:1–13.

Seabright, M. 1971. The use of proteolytic enzymes for mapping structural rearrangement in chromosomes in man. Lancet **2**:971–972.

Seargeant, L. E., and R. A. Stinson. 1979. Evidence that three structural genes code for human alkaline phosphatases. Nature **281**:152–154.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–518.

Swallow, D. M., S. Povey, M. Parker, P. W. Andrews, H. Harris, B. Pym, and P. Goodfellow. 1986. Mapping of the gene coding for the human liver/bone/kidney isozyme of alkaline phosphatase to chromosome 1. Ann. Hum. Genet. **50**:229–235.

Vockley, J., M. P. D'Souza, C. J. Foster, and H. Harris. 1984*a*. Structural analysis of human adult and fetal alkaline phosphatases by cyanogen bromide peptide mapping. Proc. Natl. Acad. Sci. USA **81**:6120–6123.

Vockley, J., L. J. Meyer, and H. Harris. 1984*b*. Differentiation of human adult and fetal intestinal alkaline phosphatases with monoclonal antibodies. Am. J. Hum. Genet. **35**:987–1000.

Weiss, M. J., P. S. Henthorn, M. A. Lafferty, C. Slaughter, M. Raducha, and H. Harris. 1986. Isolation and characterization of a cDNA encoding a human liver/bone/kidney-type alkaline phosphatase. Proc. Natl. Acad. Sci. USA **83**:7182–7186.