Structure of the Human Liver/Bone/Kidney Alkaline Phosphatase Gene*

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Mitchell J. Weiss‡§, Kunal Ray‡, Paula S. Henthorn‡¶‖, Bruce Lamb†, Tom Kadesch†, and Harry Harrist**

From the ‡Department of Human Genetics and the ¶Howard Hughes Medical Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

In man, there are multiple forms of alkaline phosphatase encoded by at least three homologous genes: placental, intestinal, and liver/bone/kidney. This report describes the characterization of the human liver/bone/kidney alkaline phosphatase locus. The gene appears to exist as a single copy in the haploid genome and is comprised of 12 exons distributed over more than 50 kilobases. In liver, kidney, SAOS-2 human osteosarcoma cells, and cultured fibroblasts, there is a single major start for transcription situated about 25 nucleotides downstream of an A/T-rich motif. The promoter region is extremely G/C-rich, is relatively abundant in the dinucleotide CpG, and contains four copies of the consensus sequence for SP1 binding (GGGCGG). The liver/bone/kidney alkaline phosphatase gene is at least five times larger than the intestinal and placental alkaline phosphatase genes, mainly due to intron size differences. Intron-exon junctions occur at analogous positions in all three genes, but there is an extra non-coding exon at the 5' end of the liver/bone/kidney alkaline phosphatase gene. The relevance of our findings with respect to the evolution of the human alkaline phosphatase multigene family is discussed.

Alkaline phosphatases (ALPs)* (orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1) are a group of membrane-bound glycoproteins that hydrolyze a broad range of monophosphate esters at alkaline pH optima. In humans, there are at least three distinct forms of ALP: (placental, intestinal, and liver/bone/kidney (L/B/K ALP)) (Harris, 1982). Examination of these different ALP polypeptides (Harris, 1982) and the corresponding cDNAs (Kam et al., 1985; Millan, 1986; Henthorn et al., 1986; Weiss et al., 1986; Henthorn et al., 1987; Berger et al., 1987) has established that they are encoded by separate homologous gene loci. A placental-like ALP found in trace amounts in testis and thymus is probably the product of a fourth locus (Millan and Stigbrand, 1983; Goldstein et al., 1982; Knoll et al., 1987). It is believed that all of the human ALP genes evolved from a single ancestral gene (Harris, 1982). Placental and intestinal ALPs are closely related, exhibiting about 87% identity at the amino acid level (Henthorn et al., 1987). L/B/K ALP is more evolutionarily distant from the other human ALPs, 52 and 57% amino acid identity to placental and intestinal ALP, respectively (Harris, 1982; Weiss et al., 1985; Henthorn et al., 1987). The genes encoding intestinal ALP, placental ALP, and probably the placental-like ALP, all map to bands q34–q37 of human chromosome 2 (Martin et al., 1987; Griffin et al., 1987), while the L/B/K ALP gene maps to the distal short arm of human chromosome 1, bands p34–p36.1 (Smith et al., 1988).

Each form of human ALP exhibits a characteristic pattern of tissue distribution (McComb et al., 1979). Placental and intestinal ALPs are found predominantly in placenta and small intestine, respectively. In contrast, L/B/K ALP is expressed in numerous tissues, including those for which it is named. Slight variations in thermostability and electrophoretic mobility between the liver/bone/kidney-type ALPs found in different tissues have been attributed to differences in post-translational modification (Harris, 1982; Moss and Whitaker, 1985).

The physiological role of ALP is unknown except that the bone isoenzyme is believed to play a role in normal skeletal mineralization (Robison, 1952; McComb et al., 1979; Wuthier and Register, 1986). Evidence for this role is provided by the rare genetic disease hypophosphatasia (Rathbun, 1948; Fraser, 1957; Rasmussen, 1983). Affected patients suffer from rickets or osteomalacia and a deficiency of L/B/K ALP in all tissues (placental and intestinal ALP levels are unaffected). The most severe form of the disease, usually lethal in infancy, is inherited as an autosomal recessive trait. The genetic defects that result in hypophosphatasia may involve mutations at the L/B/K ALP locus, although this remains to be proven.

The physiological regulation of L/B/K ALP expression has been most closely examined in bone cells. ALP is a differentiation marker for the osteoblastic phenotype and an indicator of bone formation and turnover (Rodan and Rodan, 1984). In vivo, levels of osteoblast and chondrocyte ALP increase markedly at sites where mineralization is actively occurring (McComb et al., 1979). In cultured osteosarcoma cells, ALP expression is modulated by growth conditions and various biological agents including glucocorticoids, vitamin D, and parathyroid hormone (Rodan and Rodan, 1984).
Direct examination and comparison of the genes that encode the various human ALPs should further elucidate the structure and evolutionary history of this multigene enzyme family. Examination of the promoter regions of the ALP genes may provide insight into mechanisms that mediate their characteristic patterns of tissue expression. To address these issues and to facilitate the study of hypophosphatasia, we report here an investigation of the human L/B/K ALP locus. The structure of the intestinal and placental ALP genes are presented in accompanying papers (Henthorn et al., 1988; Knell et al., 1988); comparison with the L/B/K ALP gene supports the hypothesis that the human ALP multigene family arose by a series of duplications from a common ancestor.

MATERIALS AND METHODS

RESULTS

The Human L/B/K ALP Gene Locus—A map of the L/B/K ALP locus was constructed from overlapping genomic DNA fragments (Fig. 1A). All genomic fragments which are detected by the full-length cDNA in Southern blots (Fig. 1B) are accounted for in this map, suggesting that the locus exists as a single copy. The gene consists of 12 exons distributed over more than 50 kb. Exon sequences were first localized by hybridization of radiolabeled L/B/K ALP cDNA to restriction digests of cloned genomic DNAs. Intron-exon boundaries were precisely defined by DNA sequence analysis of appropriate genomic fragments (Fig. 2 (in the Miniprint) and Table I). The sequences at the 5' and 3' ends of each intron are in agreement with the consensus sequence for intron-exon boundaries of other eukaryotic genes (Green, 1986; Mount, 1982). All introns begin with the dinucleotide GT and end with AG. Intron number 1, at least 25 kb in length, interrupts the 5'-untranslated sequence 105 bp upstream of the initiation methionine codon. All other introns interrupt the gene within protein coding regions. Exon 12, about 1025 bp, contains 263 nucleotides of coding sequence, the termination codon, and the entire 3'-untranslated region.

At the end of exon 12, there are putative 3'-mRNA processing signals (shown in Table I) that are commonly found in other eukaryotic genes (Birnstiel et al., 1985); the mRNA cleavage/polyadenylation site is flanked by the sequence AA-TAAA about 12 bp upstream, and a G/T-rich region about 12 bp downstream.

L/B/K ALP Genomic Sequences—The nucleotide sequence of each exon was determined in full. There are 11 positions where the L/B/K ALP gene exon sequences differ from our published sequence of the cDNA (Weiss et al., 1986). These discrepancies are summarized in Table II (numbered according to the convention used to describe the cDNA). Four of the eleven differences, at cDNA positions 1712–1713, 1944, 2573, and 2597, identify errors in the reported cDNA sequence. One of these errors occurs within the protein coding region, replacing a leucine for a valine at amino acid position 496. The correct sequence at nucleotides 2204–2207 in the 3'-untranslated region is unreported since the two strands of the cDNA yield ambiguous results at a single base position after resequencing in the presence of deoxy-7-deazaguanosine to alleviate compression artifacts (Mizusawa et al., 1986).

There are six authentic differences between the cDNA and genomic exon sequences. Two of these differences occur within protein coding regions: a T to C transition at position 506 represents a silent mutation, and a G to A transition at nucleotide position 880 changes a glycine codon in the cDNA to a glutamic acid codon in the genomic DNA sequence. This difference at base 880 may represent a reverse transcriptase error introduced during cDNA cloning, or a null allele in SAOS-2 cells (see below). Differences at positions 1718, 1816, 1982, and 2104 occur within the 3'-untranslated region and may represent either cloning artifacts or DNA sequence polymorphisms.

The nucleotide sequence at the 5' end of the L/B/K ALP gene is shown in Fig. 3. Nucleotide positions are numbered with the first base of the methionine initiation codon designated as +1; nucleotides 5' to this position are shown as negative numbers. Over 90% of this region was sequenced on both strands. The sequence of a stretch of G/C-rich DNA, base pairs −280 to −260, could not be determined on one strand. This region was read unambiguously on the complementary strand.

RNA Analysis—Northern analysis was performed on cellular RNAs from SAOS-2 cells, human fibroblasts, human liver, and human kidney using the L/B/K ALP cDNA as hybridization probe. As shown in Fig. 4A, the L/B/K ALP mRNA present in these cells and tissues is of similar size (about 2.5 kb), although the steady-state levels vary, being highest in SAOS-2 cells and lowest in liver. The L/B/K ALP mRNA levels from these sources roughly correlate with ALP enzyme activity levels (data not shown).

To ascertain the start(s) of mRNA transcription, S1 nuclease protection and primer extension analyses were performed (Fig. 4B).

For primer extension, a single-stranded segment of DNA, corresponding to nucleotides −128 to −89 in Fig. 3, was labeled with 32P at the 5' end, annealed with SAOS-2 cellular RNA, and extended with reverse transcriptase. One major extension product (73–75 bp), labeled 1, is observed. Two minor extension products (101–102 and 124–125 bp), labeled 2 and 3, respectively, are visible upon longer exposure (not shown).

For S1 protection analysis, a 5' end-labeled DNA probe, corresponding to nucleotides −642 to −128 in Fig. 3, was denatured, hybridized to a variety of cellular RNAs, and digested with S1 nuclease. One major fragment (69–74 bp) and one minor one (122–124 bp), labeled a and c, respectively, are specifically protected by RNA from SAOS-2 cells, normal human fibroblasts, and human kidney. Longer exposure of the autoradiogram revealed that these same fragments are also protected by human liver RNA (data not shown). Thus, L/B/K ALP mRNA transcription appears to start at the same sites in the four cell types examined, although the level of L/B/K ALP mRNA message varies among cell types.

The S1-protected fragment labeled b in Fig. 4B is present in all samples, including the rRNA control. This fragment, as well as primer extension product 2 in Fig. 4B, may represent artifacts caused by a stem-and-loop structure in the L/B/K ALP mRNA (see "Discussion").

The 5' ends of the L/B/K ALP mRNA, as mapped by primer extension and S1 nuclease protection, are summarized in Fig. 3. The two assays are in agreement. The 5' end of the majority of L/B/K ALP mRNA maps to within a few nucleotides centered around position −195. A minor transcription start site maps to within a few nucleotides of position −145. The start sites mapped by S1 protection are situated a few nucleotides downstream of those mapped by primer extension.

* Portions of this paper including “Materials and Methods” and Fig. 2 are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
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A

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BamHI

BgIII

EcoRI

HindIII

SstI

XbaI

BclI

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B

FIG. 1. A, the L/B/K ALP gene. The locus is represented as a thick line in the middle of the figure, transcription proceeding from left to right. Exons are shown as black rectangles. Overlapping inserts from recombinant phages, shown at the top of the figure, are labeled beginning with letters. Sections of genomic DNA which have been subcloned into plasmids are shown labeled beginning with numbers. Genomic fragments 14, 4, 1X/E3', and 8B/E5' are free of repetitive sequences (data not shown). Below the gene structure is a map for seven restriction endonucleases with fragment sizes shown in kilobases. This map was determined by digestion of cloned genomic DNA fragments and by hybridizing various probes to restriction enzyme digests of human genomic DNA. Broken lines indicate unmapped regions. The black squares indicate restriction sites that are polymorphic in the human population (see Ray et al., 1988). The asterisk indicates a BclI site which is present in the cDNA. B, Southern hybridization analysis of genomic DNA. Human genomic DNA was digested with the indicated restriction enzymes, size fractionated on a 0.8% agarose gel, transferred to a nylon membrane, and hybridized to 32P-radiolabeled L/B/K ALP cDNA. Molecular weight size markers, determined from BstEII-digested wild type λ DNA, are shown at left.
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| Exon 1 (86-142 bp) | Exon 2 (165 bp) | Exon 3 (120 bp) | Exon 4 (116 bp) | Exon 5 (175 bp) | Exon 6 (176 bp) | Exon 7 (144 bp) | Exon 8 (70 bp) | Exon 9 (135 bp) | Exon 10 (192 bp) | Exon 11 (120 bp) | Exon 12 (1024-1025 bp) |
|--------------------|----------------|----------------|----------------|----------------|----------------|----------------|---------------|---------------|----------------|----------------|-------------------|
| TCTGGCCGAG | GTG CCA G | GGA GAT G | GCC AAG | GAC GCT G | ATT GAC | TAC AAG | CTA TIG G | GTG GAA | ATC TTT | GAT T | TTTAAATAAAAACCTTCCCCGAGGA |
| gtaagg | gtatgc | gtgaag | gtgagg | gtgagt | gtgagt | gtagtt | gtgaag | gtgagt | gtgagt | gtgaga | cagagctgagtctttgtgagt |
| 12005 | 61 | 181 | 297 | 472 | 684 | 792 | 862 | 897 | 1189 | 1309 | 2687 |

Intron-exon organization of the L/B/K ALP gene

Nucleotide sequences of intron-exon junctions were determined according to the strategy in Fig. 2 (in the Miniprint). Exon sequences are shown in upper case letters; intron sequences are shown in lower case. The position of coding nucleotides that border the 5' and 3' ends of the introns are numbered above the DNA sequence with the first base of the ATG initiation codon designated as +1 (nucleotides within introns are not numbered). Amino acid codons bordering the splice junctions are shown numbered with the first amino acid in mature L/B/K ALP designated as +1. Exon sizes and approximate intron sizes are indicated. The region of mRNA cleavage/polyadenylation is at the 3' end of exon 12 is shown at the bottom of the figure. Nucleotides not present in the cDNA, which are 3' to the polyadenylation site, are shown in lower case. Putative 3'-mRNA processing signals, common to many other eukaryotic genes, are underlined.
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TABLE II
Differences between genomic and cDNA sequences

| Location | Position | cDNA | Genomic | Comments |
|----------|----------|------|---------|----------|
| Exon 5   | 506      | AGT  | AGC     | All sequences unambiguous |
| Exon 7   | 880      | GGG  | GAG     | All sequences unambiguous |
| Exon 12  | 1712-1713| CTC  | CTG     | cDNA sequencing error |
| Exon 12  | 1718     | C    | T       | All sequences unambiguous |
| Exon 12  | 1944     | G    | GG      | cDNA sequencing error |
| Exon 12  | 1983     | C    | A       | All sequences unambiguous |
| Exon 12  | 2104     | G    | C       | All sequences unambiguous |
| Exon 12  | 2204-2207| GCG  | GGCC    | Resequencing cDNA with 7-Deaza-dGTP: one strand reads "GGCC," the other reads "GCCG" |
| Exon 12  | 2373     | T    | C       | cDNA sequencing error |
| Exon 12  | 2397     | T    | C       | cDNA sequencing error |

FIG. 3. The 5'-end of the L/B/K ALP gene. The nucleotide sequence is oriented with transcription proceeding from left to right and numbered with the first base of the ATG initiation codon designated as +1. Nucleotides 5' to this position are indicated by negative numbers, disregarding positions within the first intron. Nucleotides within the 5' flanking region and first and second exons are shown in upper case letters; those within the first intron are shown in lower case. The major starts of transcription initiation are shown as large squares; those above the line were mapped by primer extension, those below, by S1 nuclease protection. Similarly, the smaller squares indicate a minor transcription start site. As discussed in the text, primer extension product 2 and S1 protected fragment b in Fig. 4B, whose 5' ends map to a region around nucleotide position -225, may not represent a true transcription start site and, therefore, are not shown. Four copies of the core consensus for SP1 binding are underlined. A TATA box is doubly underlined. Multiple copies of an imperfect purine-rich repeat are outlined. An 11-bp direct repeat flanking the 5' flanking region and first exon is shown by arrows. A 15-bp direct repeat within the 5' flanking region is shown by broken arrows.

form the plasmid pSV2Aalp, and ALP activity was determined after transfection into COS cells. Initial attempts to detect ALP activity above background failed. We thought it possible that the inability to express ALP was due to the mutation discussed above at position 880 of the cDNA. We therefore replaced a segment of the coding sequence in pSV2Aalp containing a guanosine residue at position 880 with a homologous segment from a different cDNA isolate which contained an adenosine residue at this position (as found in the genomic sequence). This modified plasmid (pSV2Aalp') is capable of expressing ALP enzymatic activity; in two separate experiments, COS cells transfected with pSV2Aalp' expressed ALP activity levels over 20-fold above background (data not shown). This result indicates that active L/B/K ALP contains a glutamic acid at amino acid position 218, and not glycine as previously reported (Weiss et al., 1986). Hence, this glutamic acid residue is conserved in human placental, intestinal, and L/B/K ALP and Escherichia coli ALP (see Henthorn et al. (1987) and Weiss et al. (1986)), as well as the liver/bone/kidney-type ALPs found in rat (Thiede et al., 1987), mouse (Terao and Mintz, 1987), and cow (Garattini et al., 1987). NIH 3T3 cells and CV-1 cells transfected with pSV2Aalp' stain positively for histochemical ALP (not shown), indicating that the transiently expressed enzyme resides on the cell surface. Because of the ease and economy with which ALP can be detected, we are exploring the use of ALP genes as reporters for promoter studies.

DISCUSSION

In higher organisms there are multiple forms of ALP, each exhibiting characteristic patterns of tissue distribution at different stages of development. Complementary DNAs encoding each of the major forms of human ALP have recently been isolated and sequenced (Kam et al., 1985; Millan, 1986; Henthorn et al., 1986; Weiss et al., 1986; Henthorn et al., 1987; Berger et al., 1987). These studies have confirmed the existence of at least three separate ALP genes. Direct examination of these genes provides the next step toward understanding the structure, evolution, and regulation of the various ALPs. This report describes the gene encoding the liver/bone/kidney form of human ALP. Characterization of the human intestinal and placental ALP genes are presented in
**FIG. 4. A**, Northern blot analysis of L/B/K ALP mRNA. Cellular RNAs from various sources were fractionated on a 1.5% Agarose gel with 2.2 M formaldehyde, transferred to a nylon membrane, and hybridized to $^{32}$P-labeled L/B/K ALP cDNA. RNAs are indicated from left to right: S, SOAS-2 total cellular RNA, 0.5 µg; K, human kidney total cellular RNA, 7 µg; F, human fibroblast total cellular RNA, 20 µg; L, human liver polyadenylated RNA, 7 µg. The positions of RNA molecular size markers are indicated.

**B**, mapping the 5' end of L/B/K ALP mRNA. A map of the 5' end of the liver/bone/kidney gene is shown in the middle of the figure, numbered according to the convention in Fig. 3. S1 nuclease protection and primer extension experiments are bracketed and indicated by S1 and PE, respectively. The S1 nuclease protection experiment is shown at the top of the figure and is described in the text. A DNA sequencing ladder is included for molecular sizing.

Prior to S1 nuclease digestion, radiolabeled probe was incubated with various amounts of human cellular RNAs indicated in the autoradiogram as follows: S, SAOS-2, 5 µg; L, liver, 100 µg; K, kidney, 50 µg; F, fibroblast, 50 µg; C, control, yeast tRNA, 100 µg. The RNA concentrations of all samples were normalized to 100 µg with yeast tRNA. The DNA fragments which were protected from S1 nuclease digestion (labeled a, b, and c), and the undigested probe are indicated in the autoradiogram and shown in schematic form. $^{32}$P-labeled ends are marked by an asterisk. The primer extension experiment is shown at the bottom of the figure and is described in the text. Radiolabeled primer was annealed to 10 µg of SAOS-2 cellular RNA and incubated with and without AMV reverse transcriptase (RT). Extended products 1, 2, and 3 are labeled in the autoradiogram and indicated as thick lines in the schematic diagram (products 2 and 3 are visible after longer exposure of the autoradiogram (not shown)). The primer is symbolized as an open box. Molecular sizes of extended products were determined by a DNA sequencing ladder (not shown).

**The Liver/Bone/Kidney Alkaline Phosphatase Gene**

All Liver/Bone/Kidney-type ALPs Are the Product of a Single Gene—Various liver/bone/kidney-type ALPs from different tissues display characteristic differences in thermostability and electrophoretic mobility. Based on a comparison of Southern blots and cloned genomic DNAs, it appears that the L/B/K ALP locus exists as a single copy in the haploid human genome. L/B/K ALP mRNA from SAOS-2 osteosarcoma cells, fibroblasts, liver and kidney has the same major 5' end and is processed to the same size, consistent with the hypothesis that liver/bone/kidney-type ALPs found in different tissues are all products of the same gene and contain the same polypeptide moiety (Harris, 1982; Moss and Whitaker, 1985).

The 5' End of the L/B/K ALP Gene—The start of transcription of L/B/K ALP mRNA has been mapped by S1 protection and primer extension. There appears to be a single major start site and at least one minor one (Fig. 3) which are...
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FIG. 5. Functional analysis of the human L/B/K ALP promoter region in SAOS-2 osteosarcoma cells. Cells were transfected with the indicated plasmids (described in the text) and chloramphenicol acetyltransferase activity, indicated by the faster migration of this transfection was performed in duplicate.

the same in SAOS-2 cells, fibroblasts, kidney, and liver. One S1-protected fragment (b, Fig. 4) is present in all samples including the yeast tRNA control and, therefore, may not represent a true start of transcription. The 3′ end of this protected fragment maps to an A/T-rich region centered around nucleotide −225. There is a potential stem-and-loop structure (AG = −29.6 kcal/mol) in this region at positions −241 to −212. Formation of a cruciform structure between L/B/K ALP mRNA, and the end-labeled probe would render the loop within the probe (positions −224 to −226) sensitive to S1 nuclease digestion. It is also possible that primer extension product 2, whose junctions at identical positions within the triplet codons (data not shown), further supporting the hypothesis that members of the human ALP multigene family are the product of a series of duplications of a single ancestral gene (Harris, 1982).

The first exon of the L/B/K ALP gene, occurring in the 5′-untranslated region, does not have an analogous counterpart in the intestinal and placental ALP genes. This noncoding exon and the adjacent L/B/K ALP gene promoter may have been acquired as a distinct genetic unit, analogous to functional domains of certain proteins that are encoded by separate exons (Gilbert, 1978). If so, the acquisition of this genetic unit during ALP evolution may have conferred the widespread pattern of tissue expression that is characteristic of L/B/K ALP and in marked contrast to the much more tissue specific expression of intestinal and placental ALP. The existence of a noncoding “regulatory exon” has been proposed in the growth hormone/prolactin gene family (Cooke and Baxter, 1982). It has been suggested that 10–14-bp direct repeats flanking the promoter and first exon of each of these genes may be remnants of a DNA transposition event. Similarly, there is an 11-bp direct repeat flanking the promoter and first exon of the L/B/K ALP gene (shown in Fig. 3).
The promoter region of the L/B/K ALP gene is quite distinct from the intestinal and placental ALP gene promoters. The L/B/K ALP gene contains a G/C-rich promoter and a CpG island at its 5' end. These features have been observed in many other genes that exhibit a widespread tissue distribution, most notably the "housekeeping" genes (Serfling et al., 1985; Bird, 1986; Dynan, 1986). In contrast, the intestinal and placental ALP genes, which are highly tissue-specific, contain promoters that are much less G/C-rich and are not associated with CpG islands (Henthorn et al. (1988) and Knoll et al. (1988), accompanying articles).

Although the L/B/K ALP gene resembles the housekeeping genes in its promoter structure and widespread tissue distribution, it is not completely accurate to apply this term to the L/B/K ALP gene. Housekeeping implies that the gene encodes an enzyme which performs an essential metabolic function (Dynan, 1986); the function for L/B/K ALP in most tissues is unknown. The only biological role which has been established for ALP is one in bone mineralization, a tissue-specific function. Furthermore, there is a marked variation in L/B/K ALP expression in different cell types. Most notably, L/B/K ALP is expressed at high levels in mineralizing chondrocytes and osteoblasts (McComb et al., 1979). Hence, the L/B/K ALP gene displays characteristics of both housekeeping and tissue-specific genes.

**Intron-Exon Organization of the ALP Genes**—A diagram of the relationship between L/B/K ALP gene and protein primary structure is shown in Fig. 6. Except for the first exon (discussed above), this diagram also applies to the intestinal and placental ALP genes. The signal peptide along with the first three amino acids of human liver/bone/kidney, intestinal, and placental ALP are encoded by a separate exon. Contrary to the prediction of Kam et al. (1985), the carboxy-terminal stretch of hydrophobic amino acids encoded by the alp cDNAs, which presumably participate in membrane localization, are not represented by a separate exon.

Further analysis of the relationship between ALP protein structure and intron-exon organization is impeded by a lack of structural information on the mammalian ALPs. However, some observations can be made by considering the structure of *E. coli* ALP, which has been determined to a high degree of resolution by crystallographic studies (Sowadski et al., 1985; Wyckoff et al., 1983). Although the *E. coli* and human ALP polypeptides demonstrate only about 25% positional identity in an optimal alignment, the amino acid sequences that comprise the active pocket are highly conserved (Kam et al., 1985; Millan, 1986; Weiss et al., 1986). This active pocket consists of several components distributed throughout the polypeptide chain: three functional metal binding sites located near the carboxyl ends of four parallel β-sheet strands and the amino end of an antiparallel β-sheet, a reactive serine linked to the amino end of an α-helix, and an arginine residue thought to stabilize the transition state during catalysis. Localization of these regions within the human ALP genes (Fig. 6) reveals that the enzyme active pocket is formed by structural elements from six separate exons.

Localization of introns within the three-dimensional structure of *E. coli* ALP, based upon alignment of homologous regions of the bacterial and human polypeptide sequences (Sowadski et al., 1985; Weiss et al., 1986), suggests that the splice junctions coincide with regions at the surface of the enzyme and tend to fall in between stretches of α-helix or β-sheet units of secondary structure. These trends have been noted in other genes which encode globular proteins (Lonberg and Gilbert, 1985; Craig et al., 1982; Blake, 1986). Confirmation of these observations awaits detailed structural analysis of mammalian ALPs.

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METHODS

Materials and General Methods—Except where noted otherwise, standard techniques were used for the isolation and manipulation of DNA and RNA (Maniatis et al., 1982). Nucleic acid modification enzymes were purchased from Bethesda Research Laboratories (Catonsville, MD). Restriction endonuclease were purchased from New England Nuclear ( Beverly, MA). and Boehringer Mannheim (Mannheim, FRG). and were used according to the manufacturers instructions. All other chemicals were purchased from Sigma Chemical (St. Louis, MO). All experiments were performed using dithiothreitol-saturated DNA (Ginn, B. T., and Gaddum, J. H. 1947). A 10% stock solution of cold tritiated water (95 S) was used to generate tritiated water (100 S). Each experiment was performed on duplicate wells with a DNA concentration of 100 S. To label DNA, the reaction mixture was heated to 95°C for 5 min and allowed to cool slowly to room temperature. The labeled primer was extended with AMV reverse transcriptase for 5 min at 37°C to generate labeled primer, precipitated, and analyzed on a 5% polyacrylamide gel after electrophoresis.

The primers for S1 mapping were synthesized by an oligo primer synthesizer (Oligo Synthesizing Machine, DNA Sequencing Research, New York, NY). The oligo primer synthesizer was programmed to generate labeled primer (95 S) that was used to generate tritiated water (100 S). To label DNA, the reaction mixture was heated to 95°C for 5 min and allowed to cool slowly to room temperature. The labeled primer was extended with AMV reverse transcriptase for 5 min at 37°C to generate labeled primer, precipitated, and analyzed on a 5% polyacrylamide gel after electrophoresis.

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