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VITAMIN D BINDING PROTEIN: GENOMIC STRUCTURE, FUNCTIONAL DOMAINS, AND mRNA EXPRESSION IN TISSUES

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Summary—The vitamin D binding protein (DBP), alternatively known as Gc-globulin, is a member of the albumin (ALB) and α-fetoprotein (AFP) gene family. The rat DBP gene is expressed at high levels in liver and at moderate levels in kidney, testis, abdominal fat, and yolk sac. Very low levels of DBP as well as ALB and AFP transcripts can be detected in all other tissues studied by the reverse transcriptase/polymerase chain reaction technique. During development, liver DBP gene transcripts are detectable at 14 days of gestation and levels rise gradually until adulthood in parallel with ALB. DBP present on the surface of U937 monocyte-derived cells is acquired from serum, suggesting cell surface binding sites for DBP. The rat DBP gene has been cloned and characterized. It spans 35 kb and contains 13 exons and 12 introns. The DBP gene contains two fewer exons than the ALB or AFP genes, accounting for the shortest size of its mRNA and protein product. Its 5′-flanking region contains a high degree of structural similarity to both ALB and AFP promoters.

INTRODUCTION

Vitamin D binding protein (DBP), also known as Gc-globulin, is an abundant serum protein [1]. Unlike the other steroid hormone binding proteins in mammals, DBP circulates at a striking 30-fold molar excess with respect to its vitamin D sterol ligands [2]. As a consequence, only 2% of its carrier sites are ever occupied, suggesting that DBP might have additional functions to account for its excess in plasma. A number of these additional functions have been identified. DBP sequesters globular actin, preventing it from polymerizing into actin fibrils [3]. In this role it serves as a major component of the plasma actin scavenger system [4]. In addition, DBP binds to complement C5a and enhances the effects of complement activation on neutrophil chemotaxis [5, 6]. DBP binds and may contribute to the plasma transport of unsaturated fatty acids, a role also attributed to the related albumin (ALB) molecule [7]. DBP binds to the endotoxin of *Esherichia coli*, but a functional role with respect to this binding has not been elucidated [8]. DBP has been detected on the surface of peripheral blood monocytes, some lymphocytes, placental trophoblasts, and the rat yolk sac endoderm [9–12]. Although its function on the cell surface is unknown, it has been postulated to facilitate vitamin D sterol transfer into the cell, or transduce cell surface signals in conjunction with the cytoskeleton. Extensive worldwide screening has detected a DBP null allele but no DBP null homozygotes [13], suggesting that one of its above functions, or an unappreciated function is critical to survival. This is in marked contrast to the related ALB molecule in which analbuminemia does not appear to cause disease [14].

We have previously determined the structure of both rat and human DBP mRNAs from cloned cDNAs [15, 16]. The predicted amino acid sequence is shown in Fig. 1. DBP was noted to have a repeating pattern of cysteine residues, nearly identical to the cysteine residue patterns in ALB and α-fetoprotein (AFP). DBP was also found to have about 22% amino acid and 40% nucleotide identity to ALB and AFP. It was noted that DBP contained three internally homologous domains similar to the three internal domains present in ALB and AFP. However, DBP's third internal domain terminated 124 amino acids prematurely resulting in a smaller protein. The molecular basis for this truncation

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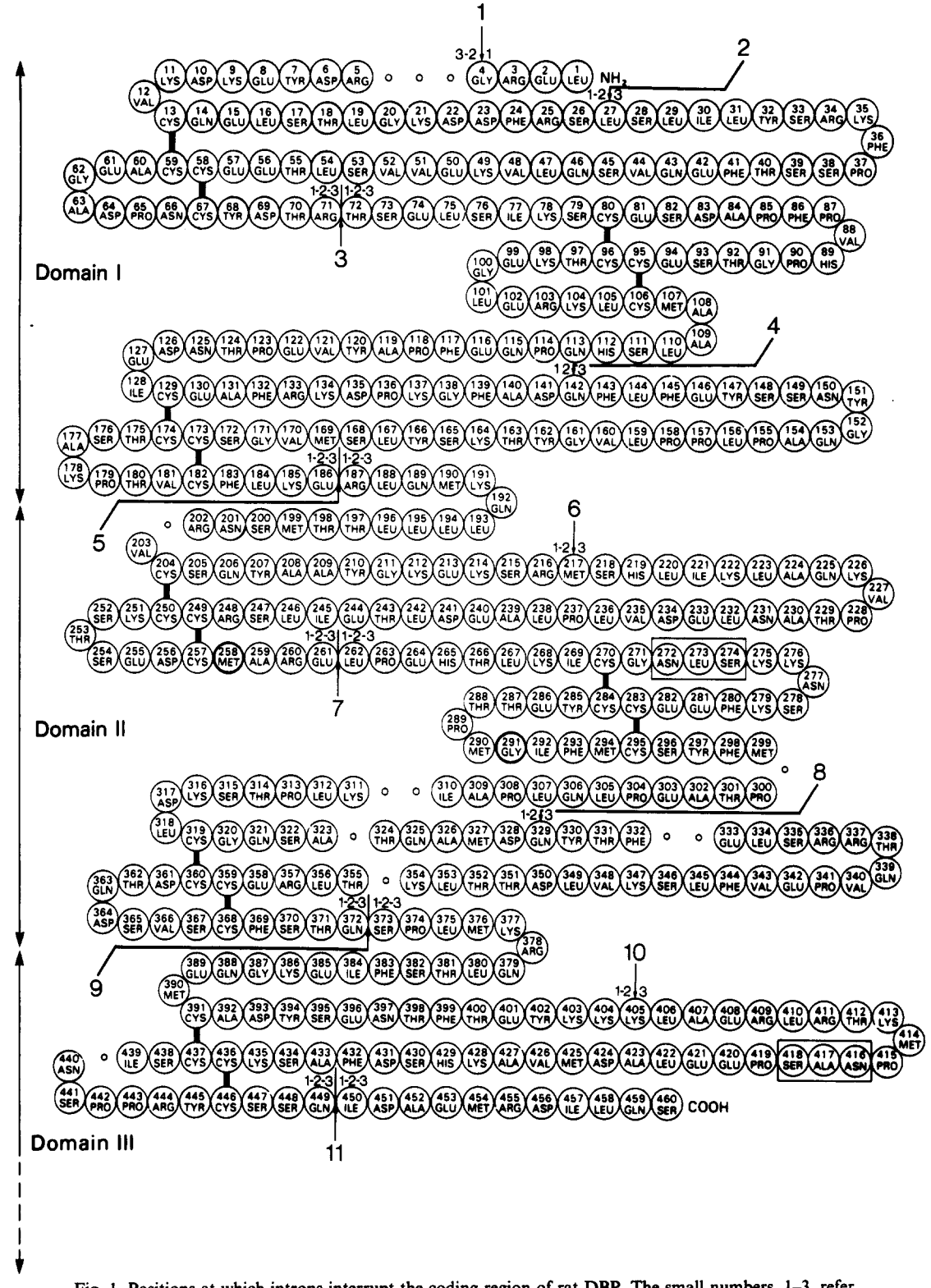

Fig. 1. Positions at which introns interrupt the coding region of rat DBP. The small numbers, 1–3, refer to base positions within a codon. Note that introns 3, 5, 7, 9, and 11 occur between codons, but introns 2, 4, 6, 8, and 10 interrupt bases 2 and 3 of the codon. This alternating pattern of intron positions is conserved in the ALB and AFP genes as well. Only the positions of intron 1, which is the most divergent in the multigene family, does not follow this pattern.

was unknown. All three genes were found to be encoded in the human chromosome 4q11–q22 region, and to be syntenic on rat chromosome 14 [17, 18]. We proposed that they constituted a multigene family and that the DBP gene duplication predated the more recent ALB and AFP duplication.

EXPERIMENTAL

Many of the materials and methods have been described in detail elsewhere: the cloned rat DBP (rDBP) cDNA used as probe [15], Northern analysis and the reverse transcriptase/polymerase chain reaction (RT/PCR) approach and its controls, cloning of kidney DBP cDNA [19], culture of U937 cells, preparation and characterization of the anti-DBP F(ab')2 fragments, immunogold electron microscopy and flow-activated cell sorting to detect cell surface DBP [20]. The details of the cloning and DNA sequencing of the rDBP gene has been described in detail elsewhere [21]. Briefly a Sprague–Dawley rat liver partial EcoRI library in lambda Charon 4A [22] was screened with rDBP cDNA. Two genomic clones which did not span the entire gene were isolated. To obtain the remainder of the gene, a second EcoRI genomic library was generated using the Lambda–DASH phage cloning vector (Stratagene, La Jolla, Calif.) and was screened. By comparing restriction endonuclease maps of Sprague–Dawley total genomic DNA to maps of the cloned gene, it was concluded that the entire rDBP gene was cloned. The rDBP gene was subcloned into the vector pTZ18R (Pharmacia). DNA sequence analysis was done on both double- and single-stranded templates by the chain termination method [23] using the genomic subclones in the vector pTZ18R and M13K07 helper phage in the *E. coli* host cell line NM522. Computer-based DNA sequence analysis utilized IBI/pustell software, and database searches utilized Intelligenetics sequence analysis software on a Sun Workstation.

RESULTS AND DISCUSSION

DBP gene expression

The DBP gene, like those for other serum proteins, was initially believed to be expressed only in the liver. To determine if this was true, RNA was isolated from a series of rat tissues and transferred to Northern blots. These blots were hybridized to a DBP cDNA probe. A 1.8 kb DBP mRNA was visible in the liver lane after a 2 h exposure, but identically sized bands were easily visible in testis, kidney, yolk sac, placenta, and fat lanes only after a 2 week exposure. No DBP mRNA was detected by this approach in heart, brain, spleen, thymus, or gut [19]. Intrigued by the presence of DBP mRNA in kidney, the site of conversion of $25(OH)D_3$ to the active $1,25(OH)_2D_3$ form, we wondered whether a unique form of DBP might serve a local vitamin D sterol transport role in this tissue. Therefore a DBP cDNA was cloned from rat kidney mRNA and sequenced. Its sequence is identical to the cloned rDBP cDNA from liver with the exception of a few polymorphic base substitutions. Placental DBP mRNA was also surveyed by sizing overlapping fragments generated by RT/PCR and all were found to be identical in size to comparably generated liver DBP RT/PCR fragments [19]. Therefore, we conclude that there is only one form of DBP mRNA which is expressed in rat liver, kidney, and placenta.

Since the detection of mRNA is a function of the sensitivity of the technique used, we extended our survey of DBP gene expression by applying the more sensitive reverse transcriptase/polymerase chain reaction (RT/PCR) technique, modified so that the 5'-oligonucleotides were end-labeled with ^{32}P. Surveys with oligonucleotides specific for ALB and AFP sequences were done in parallel. Our semi-quantitative analysis of RT/PCR data has been described elsewhere [19]. Starting with the same RNA samples used for Northern analysis, DBP transcripts were detected in the same tissues as by Northern analysis after 20 cycles (Table 1). ALB transcripts were detected in liver, spleen, kidney, testes, and yolk sac/placenta, and AFP transcripts in yolk sac/placenta, brain, uterus, and testes. However after 32 cycles, DBP, ALB, and

Table 1. Tissue distribution of DBP, AFP, ALB steady-state transcripts by RT/PCR analysis

Transcript level	DBP	AFP	ALB
High	Liver	Yolk Sac	Liver
Low	Kidney	Uterus	Uterus
	Testis	Testis	Testis
	Placenta	Placenta	Placenta
	Yolk sac	Brain	Yolk sac
	Fat	Liver	
Very low	Lung	Lung	Lung
	Heart	Heart	Heart
	Gut	Gut	Gut
	Spleen	Spleen	Spleen
	Uterus	Kidney	Kidney
	Brain	Fat	Fat
			Brain

AFP transcripts were detectable in every tissue examined (Table 1). In parallel control experiments, a sample including all reagents except RT resulted in no bands on the gel, and oligonucleotides specific for insulin detected transcripts in pancreas, spleen, and faintly in thymus after 32 cycles. The remaining tissues were negative. There are two possible explanations for this finding: expression of these genes in a subset of cells present in each tissue or a low level leakiness of transcription of these genes in all tissues. The latter was concluded for the similar ubiquitous expression of dystrophin and several other genes [24, 25].

The ontogeny of DBP gene expression in livers and yolk sacs obtained from timed pregnant rats was studied by Northern analysis and compared to that for the ALB and AFP genes. All three genes are expressed during fetal life. AFP is present most abundantly in the yolk sac during fetal life while ALB and DBP are most abundant in the liver during adult life. DBP and ALB gene expression are first detectable at 14 days of gestation, and rise in parallel until adulthood. Rat AFP levels remain fairly low and constant in the liver throughout fetal and neonatal life, but peak in the yolk sac at 17 days of gestation. DBP parallels AFP levels in yolk sac peaking at 17 days gestation, although at lower levels. ALB is not detectable in the yolk sac. We conclude that DBP shares features of both ALB and AFP regulation during development.

Cell surface DBP

Like cortisol binding globulin, sex hormone binding globulin [26, 27], and ALB [28], human DBP has been detected on the surface of cells: specifically peripheral blood monocytes, some lymphocytes, and placental cells [9–12]. In addition, DBP has been detected on the surface of the human monocyte-derived U937 cell line. To determine whether cell surface DBP arises from the expression of the DBP gene within the cell or is acquired by the cell surface from the media bathing the cells, we undertook the following experiments in collaboration with Dr John Haddad's laboratory at the University of Pennsylvania [20]. DBP-free serum was generated by actin-affinity chromatography, and U937 cells were grown in its presence or absence. Cells in each group were studied by immunogold electron microscopy and fluorescence-activated flow cytometry using anti-DBP $F(ab')_2$ fragments for the immunodetection. As seen in Table 2, the average number of gold grains per thin-sectioned U937 cell fell from 5.62 to 0.26 after cultivation in DBP(−) media. Similarly the number of positive cells detected by flow cytometry (FACS) fell from more than 95% to less than 22% under the same conditions. U937 cells, resting and stimulated primary T and B lymphocytes, and placental tissues were subjected to Northern analysis. No human DBP(hDBP) mRNA was detectable in any of these samples, but was detectable in control Hep 3B mRNA. U937 cell RNA was studied with the more sensitive RT/PCR approach and after 25 cycles of PCR and 10 days of exposure, no DBP transcripts were detectable while a parallel experiment using Hep 3B mRNA as template was positive. We conclude that U937 cell surface DBP decreases in the absence of serum and that no evidence for DBP gene expression is detectable in U937 cells. Therefore these cells, and by extension perhaps others, acquire DBP from serum. This suggests the presence of DBP binding sites on the cell surface as have been described previously for ALB.

DBP genomic structure

To further understand the molecular basis for DBP gene expression, we have cloned the full-length rDBP gene [21]. Four genomic EcoRI clones spanning the gene were isolated and characterized by restriction mapping. All exon/intron boundaries and flanking regions were sequenced. The gene is quite large, spanning 35 kb of DNA. It contains 13 exons and 12 introns. Each intron begins and ends with the characteristic splice-donor and splice-acceptor

Table 2. Cell surface DBP levels fall in the absence of serum DBP

Media	$F(ab')_2$	Gold particles per cell slice	FACS positive cells
DBP (+)	Anti-DBP	5.62 (±0.20)	>95.0%
	Nonimmune	0.04 (±0.04)	<1.2%
DBP (−)	Anti-DBP	0.26 (±0.05)	<22.0%
	Nonimmune	0.04 (±0.00)	<1.2%
DBP (−) supplemented with bDBP	Anti-DBP	N.D.	>85.0%

consensus sequences [29], and each intron interrupts the DBP coding sequence at positions nearly identical to those predicted based upon the similarity of the DBP cDNA sequence to those of ALB and AFP [15]. The actual intron positions are indicated in Fig. 1. The rDBP gene is considerably larger than the 20 kb rAFP [30] and 15 kb rALB genes [31]. This difference in size is largely due to the first intron of DBP which contains 10.5 kb of sequence. The numbers of coding bases in exons 1 and 2 of each of the three genes are also unique. This divergence was anticipated because the amino termini of all three proteins differs markedly in their first 52 amino acid residues. DBP in particular is the only member of the family that retains a disulfide bond anchoring the first loop of the first internal domain (Fig. 1).

The 3′ portion of the DBP gene also diverges from ALB and AFP, containing two fewer exons overall. This is in contrast to a marked degree of similarity in exon (and to a lesser extent intron) sizes between exons 3 through 11 of DBP, ALB, and AFP. However, DBP's terminal two exons, 12 and 13 are very similar in size to the terminal exons 14 and 15 of the ALB and AFP genes. In addition, exon 12 of DBP and exons 14 of ALB and AFP contain the translational termination codon as well as 3′ nontranslated sequences, while exon 13 of DBP and exons 15 of ALB and AFP contain only 3′ nontranslated sequences. This observation strongly suggests that the truncation of the third internally similar domain of DBP resulted from the loss of the original internal exons 12 and 13 from the DBP progenitor gene. This observation confirms the hypothesis of Gibbs and Dugaiczyk [32].

The unique structural features of the DBP gene may have functional correlations. The loss of the progenitor exons 12 and 13 in DBP deleted the region of the protein thought to encode the highest affinity fatty acid binding site in ALB. In support of this, DBP has been found to bind unsaturated fatty acids, but with fewer binding sites and at lower affinity than ALB [33]. The unique amino terminal region of the DBP molecule has been previously implicated in 25(OH)D_3 binding, a function unique to DBP in this gene family. A tryptic digestion of hDBP was covalently bound to a bromoacetoxy derivative of 25(OH)D_3. Peptide fragments bound to the sterol were subjected to amino acid sequencing. Two of the sequenced peptides were found to be derived from the amino terminus of the DBP molecule [34]. Finally, in a previous comparison of the DBP cDNA sequence to ALB and AFP cDNAs, a stretch of amino acids in the third domain of DBP was localized that shared no common residues with the other two molecules [15]. This region is largely encoded by DBP exon 10. By searching for sequence similarities to the actin binding domains of other molecules [35],

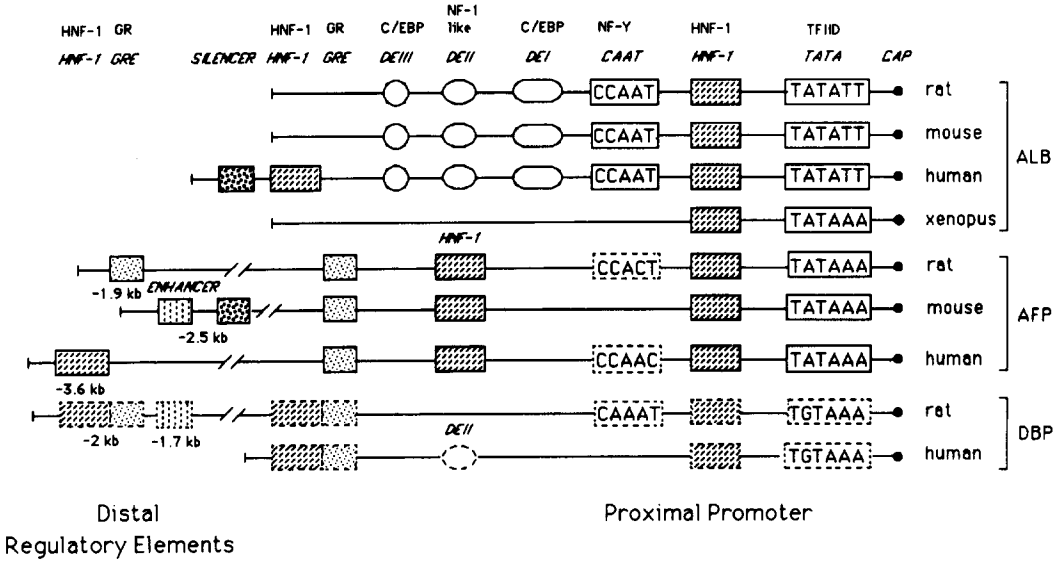

Fig. 2. Putative promoter elements in the 5′-flanking region of the DBP gene. The promoter elements of the genes whose interactions with the *trans*-acting factors are documented are indicated by solid outlines, while putative elements, based upon sequence similarity, are indicated by the dashed outlines. *Cis* elements are labeled in italics, and the *trans*-acting factors are in standard letters.

a consensus region was identified in DBP which lies completely within this unique 10th exon region. DBP is the only member of the family that contains this consensus and the only member that binds actin. Experimental verification of these functional correlations is underway.

We have compared the sequences of the 5′-flanking regions of all the members of this gene family [21]. The ALB proximal promoter has been extremely well characterized by several groups of investigators [36–39]. Functionally active *cis* elements have been identified and the protein factors binding to these elements have been purified as summarized in Fig. 2. Regulatory elements detected in the AFP gene are also indicated in this figure. The bottom two lines summarize the location of regions of sequence similarity to the ALB or AFP elements in the rat [21] and human [40] DBP 5′-flanking regions. These putative elements are indicated by dashed outlines. DBP's putative TATA box is predicted to be an unusual TGTAAA motif. Upstream of this is an HNF-1 binding site whose sequence and position are highly conserved in this multigene family, present even in *Xenopus laevis* ALB [41]. This element binds HNF-1, the factor currently thought to be responsible for liver-specific gene expression [42]. The functionally active CAAT box present in the ALB genes is only poorly conserved in AFP and DBP and may not be functional in the latter two genes. Similarity to distal element II (DEII) of the ALB promoter can be found in hDBP but not in rDBP. Features such as this might account for differences between the regulation of the rDBP and hDBP gene. Rat AFP is negatively regulated by glucocorticoids via a GRE [43] and two putative GRE's are present in rDBP in similar positions. The more proximal element occurs at approximately the same distance from the cap site as the active element in AFP. However, in DBP it is juxtaposed to a second putative HNF-1 consensus. Further upstream in rDBP this motif is repeated, as is the GRE alone in AFP. The functional significance of these elements in the DBP promoter is currently under study.

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REFERENCES

1. Cooke N. E. and Haddad J. G.: Vitamin D Binding Protein (Gc-globulin). *Endocrine Rev.* **10** (1989) 294–307.
2. Haddad J. G.: Traffic, binding and cellular access of vitamin D sterols. *Bone and Mineral Research V* (Edited by W. A. Peck). Elsevier, Amsterdam (1987) 281–308.
3. Lees A., Haddad J. G. and Lin S.: Brevin and vitamin D binding protein: comparison of the effects of two serum proteins on actin assembly and disassembly. *Biochemistry* **23** (1984) 3038–3047.
4. Haddad J. G., Harper K. D., Guoth M., Pietra G. G. and Sanger J. W.: Angiopathic consequences of saturating the plasma scavenger system for actin. *Proc. Natn. Acad. Sci. U.S.A.* **87** (1990) 1381–1385.
5. Kew R. R. and Webster R. O.: Gc-globulin (Vitamin D-binding protein) enhances the neutrophil chemotactic activity of C5a and C5a des Arg. *J. Clin. Invest.* **82** (1988) 364–369.
6. Perez H. D., Kelly E., Chenoweth D. and Elfman F.: Identification of the C5a des Arg cochemotaxin-homology with vitamin D-binding protein (group-specific component globulin). *J. Clin. Invest.* **82** (1988) 360–363.
7. Williams M. H., Van Alstyne E. L. and Galbraith R. M.: Evidence of a novel association of unsaturated fatty acids with Gc (vitamin D-binding) protein. *Biochem. Biophys. Res. Commun.* **153** (1988) 1019–1024.
8. Berger D. and Berger H. G.: Evidence for endotoxin binding capacity of human Gc-globulin and transferrin. *Clin. Chim. Acta* **163** (1987) 289–299.
9. McLeod J. F., Kowalsky M. A. and Haddad J. G.: Characterization of monoclonal antibody to human serum vitamin D-binding protein (Gc-globulin): recognition of an epitope hidden in membranes of circulating monocytes. *Endocrinology* **119** (1986) 77–83.
10. Petrini M., Emerson D. L. and Galbraith R. M.: Linkage between surface immunoglobulin and cytoskeleton of B-lymphocytes may involve Gc protein. *Nature* **306** (1983) 73–74.
11. Nestler J. E., McLeod J. F., Kowalski M. A., Strauss III J. F. and Haddad J. G.: Detection of vitamin D-binding protein on the surface of cytotrophoblasts isolated from human placentae. *Endocrinology* **120** (1987) 1996–2002.
12. Danan J. L., Delorme A. C., Ripoche M. A., Bouillon R., Van Baelen H. and Mathieu H.: Presence of immunoreactive vitamin D-binding protein in rat yolk sac endodermal cells. *Endocrinology* **117** (1985) 243–247.
13. Cleve H. and Constans J.: The mutants of the vitamin D-binding protein: more than 120 variants of the GC/DBP system. *Vox Sang.* **54** (1988) 215–225.
14. Gitlin D. and Gitlin J. D.: Genetic alterations in plasma protein. In *The Plasma Proteins* (Edited by F. W. Putnam). Academic Press, New York (1975) pp. 331–334.
15. Cooke N. E.: Rat vitamin D binding protein: determination of the full-length primary structure from cloned cDNA. *J. Biol. Chem.* **261** (1986) 3441–3450.
16. Cooke N. E. and David E. V.: Serum vitamin D-binding protein is a third member of the albumin and α-fetoprotein gene family. *J. Clin. Invest.* **76** (1985) 2420–2424.
17. Cooke N. E., Willard H. G., David E. V. and George D. L.: Direct regional assignment of the gene for vitamin D binding protein (Gc-globulin) to human chromosome 4q11-q13 and identification of an associated DNA polymorphism. *Hum. Genet.* **73** (1986) 225–229.
18. Cooke N. E., Levan G. and Szpirer J.: The rat vitamin D binding-protein gene (Gc-globulin) gene is syntenic with rat albumin and α-fetoprotein genes on chromosome 14. *Cytogenet. Cell Genet.* **44** (1987) 98–100.
19. McLeod J. F. and Cooke N. E.: The vitamin D-binding protein, α-fetoprotein, albumin multigene family: detection of transcripts in multiple tissues. *J. Biol. Chem.* **264** (1989) 21760–21769.

20. Guoth M., Murgia A., Smith R. M., Prystowsky M. B., Cooke N. E. and Haddad J. G.: Cell surface vitamin D-binding protein (Gc-globulin) is acquired from plasma. *Endocrinology* **127** (1990) 2313–2321.
21. Ray K., Wang X., Zhao M. and Cooke N. E.: Structure of the vitamin D binding protein (Gc-globulin) gene: structural analysis, functional and evolutionary correlations. *J. Biol. Chem.* **266** (1991) 6221–6229.
22. Sargent T. D., Wu J.-R., Sala-Trepat J. M., Wallace R. B., Reyes A. A. and Bonner J.: The rat serum albumin gene: analysis of cloned sequences. *Proc. Natn. Acad. Sci. U.S.A.* **76** (1979) 3256–3260.
23. Sanger F., Nicklen S. and Coulson A. R.: DNA sequencing with chain-terminating inhibitors. *Proc. Natn. Acad. Sci. U.S.A.* **75** (1977) 5463–5467.
24. Chelly J., Kaplan J. C., Maire P., Guatron S. and Kahn A.: Transcription of the dystrophin gene in human muscle and non-muscle tissues. *Nature* **333** (1988) 858–860.
25. Chelly J., Concordet J.-P., Kaplan J. C. and Kahn A.: Illegitimate transcriptions: transcription of any gene in any cell type. *Proc. Natn. Acad. Sci. U.S.A.* **86** (1989) 2617–2621.
26. Kuhn R. W.: Corticosteroid-binding globulin interactions with target cells and plasma membranes. *Ann. N.Y. Acad. Sci.* **538** (1988) 146–158.
27. Rosner R.: The functions of corticosteroid-binding globulin and sex hormone-binding globulin: recent advances. *Endocrine Rev.* **11** (1990) 80–91.
28. Schnitzer J. E., Carley W. W. and Palade G. E.: Albumin interacts specifically with a 60-kDa microvascular endothelial glycoprotein. *Proc. Natn. Acad. Sci. U.S.A.* **85** 6773–6777.
29. Mount S. M.: A catalogue of splice junction sequences. *Nucleic Acids Res.* **10** (1982) 459–472.
30. Nahon J.-L., Danan J.-L., Poiret M., Tratner I., Jose-Estanyol M. and Sala-Trepat J.-M.: The rat α-fetoprotein and albumin genes: transcriptional control and comparison of the sequence organisation and promoter region. *J. Biol. Chem.* **262** (1987) 12479–12487.
31. Sargent T. D., Jagodzinski L. L., Yang M. and Bonner J.: Fine structure and evolution of the rat serum albumin gene. *Molec. Cell. Biol.* **1** (1981) 871–883.
32. Gibbs P. E. M. and Dugaiczyk A.: Origin of structural domains of the serum-albumin gene family and a predicted structure of the gene for vitamin D-binding protein. *Molec. Biol. Evol.* **4** (1987) 364–379.
33. Calvo M. and Ena J. M.: Relations between vitamin D and fatty acid binding properties of vitamin D-binding protein. *Biochem. Biophys. Res. Commun.* **163** (1989) 14–17.
34. Robzyk P., McLeod J., Kowalski M., Cooke N. E. and Haddad J. G.: The binding domains of human plasma vitamin D binding protein. *J. Bone Min. Metab.* **4** (1989) S262.
35. Tellam R. L., Morton D. J. and Clarke F. M.: A common theme in the amino acid sequences of actin and many actin-binding proteins? *Trends Biochem. Sci.* **14** (1989) 130–133.
36. Tronch F., Rollier A., Herbomel P., Bach I., Cereghini S., Weiss M. and Yaniv M.: Anatomy of the rat albumin promoter. *Molec. Biol. Med.* **5** (1990) 173–185.
37. Maire P., Wuarin J. and Schibler U.: The role of *cis*-acting promoter elements in tissue-specific albumin gene expression. *Science* **244** (1989) 343–345.
38. Frain M., Hardon E., Ciliberto G. and Sala-Trepat J. M.: Binding of a liver-specific factor to the human albumin gene promoter and enhancer. *Molec. Cell. Biol.* **10** (1990) 991–999.
39. Jose-Estanyol M. Poliard A., Foiret D. and Danan J.-L.: A common liver-specific factor binds to the rat albumin and α-fetoprotein promoters *in vitro* and acts as a positive *trans*-acting factor *in vivo*. *Eur. J. Biochem.* **181** (1989) 761–766.
40. Yang F., Naberhaus K. H., Adrian G. S. Gardella J. M., Brissenden J. E. and Bowman B. H.: The vitamin D-binding protein gene contains conserved nucleotide sequences that respond to heavy metal, adipocyte and mitotic signals. *Gene* **54** (1987) 285–290.
41. Moskaitis J. E., Sargent T. D., Smith L. H. Jr., Pastori R. L. and Schoenberg D. R.: *Xenopus laevis* serum albumin: sequence of the complementary deoxyribonucleic acids encoding the 68- and 74-kilodalton peptides and the regulation of albumin gene expression by thyroid hormone during development. *Molec. Endocr.* **3** (1989) 464–473.
42. Q. Frain M., Swart G., Monaci P., Nicosia A., Stampfli S., Frank R. and Cortese R.: The liver-specific transcription factor LF-B1 contains a highly diverged homeobox DNA binding domain. *Cell* **59** (1989) 145–157.
43. Guertin M., LaRue H., Bernier D., Wrange O., Chevrette M., Gingras M.-C. and Belanger L.: Enhancer and promoter elements directing activation and glucocorticoid repression of the α-fetoprotein gene in hepatocytes. *Molec. Cell. Biol.* **8** (1988) 1398–1407.