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Structure and expression of the genes coding for human α1-acid glycoprotein

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α1-acid glycoprotein (αAGP) is a well-characterized human plasma protein. Its structural properties have been studied for many years but little is known about its function. Amino acid sequence analysis of purified human αAGP from plasma pooled from several individuals showed considerable heterogeneity. We have cloned the genomic DNA segment encoding αAGP and we show that it contains three adjacent αAGP coding regions, AGP-A, B and B′, identical in exon–intron organization but with slightly different coding potential. These results account for the heterogeneity observed by protein sequencing. Southern blot analysis indicates that the cloned cluster contains all the αAGP coding sequences present in the human genome. The larger majority of αAGP mRNA in human liver is transcribed from AGP-A, whose promoter and cap site have been determined while the level of AGP-B and B′ mRNA in human liver is very low. Using Hep3B hepatoma cells as a model system for the *in vitro* study of the acute phase reaction, we show that only AGP-A is strongly induced by treatment with culture medium of LPS stimulated monocytes.

Key words: α1-acid glycoprotein/plasma protein/mRNA/hepatoma cells

Introduction

Human α1-acid glycoprotein (αAGP) or orosomucoid is a plasma protein synthesized in the liver and secreted into the blood where its concentration is ~2.5 mg/ml in healthy normal individuals. It is a well-characterized glycoprotein composed of a carbohydrate and a polypeptide moiety accounting for ~45 and 55% of its 40 000 dalton mol. wt respectively (Schmid, 1975). Approximately 12% of the unusually high carbohydrate content is constituted of sialic acid.

A high degree of heterogeneity was found both in the carbohydrate portion and in the amino acid composition: (i) different linkages of sugars (in particular sialic acid to galactose) result in different patterns of electrophoretic mobilities of the protein purified to homogeneity from different individuals. Analysis of families showed genetic transmission of these patterns. (ii) Multiple amino acid substitutions were found in the protein sequence determined by Schmid (1975). The protein is a single polypeptide chain of 183 amino acids. In 21 positions of the sequence two alternative amino acids were detected. As the analysis was performed on a protein purified from plasma pooled from several individuals, it was not possible to establish unambiguously how many different sequences exist and whether the heterogeneity was due to polymorphism, to multiple gene products or to both (Schmid, 1975).

The plasma concentration of αAGP in humans increases considerably as a consequence of acute infections or inflammations, as well as in response to various other stimuli (Schmid, 1975). This phenomenon, involving several other proteins, is referred to as the acute phase reaction (Kuschner *et al.*, 1981) and the affected proteins are called acute phase reactants. This phenomenon is common to all mammals so far investigated, even though the set of acute phase reactants are not identical in the various species. The molecular mechanism of this phenomenon is unknown. There is, however, growing evidence that the hepatocyte responds to acute phase signals produced by macrophages by increasing the rate of transcription of the acute phase genes and the stability of the corresponding mRNAs (Ritchie and Fuller, 1983; Darlington *et al.*, 1986).

We have recently cloned and sequenced cDNAs and genomic segments coding for human AGP and have proposed that there are at least two different genes coding for two slightly different

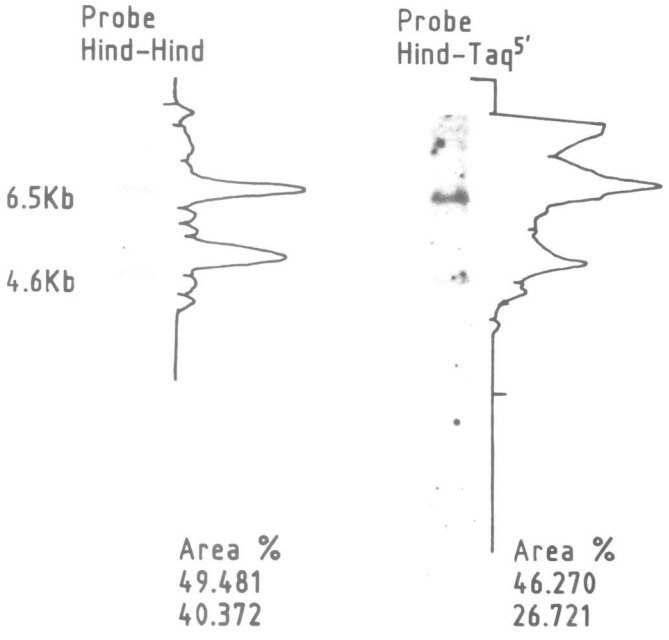

Fig. 1. Southern blot analysis of human genomic DNA from individual C.S. restricted with *Hin*dIII. The *Hind*–*Hind* probe is the purified insert of plasmid pAGP2, containing the complete cDNA of AGP-A. (Dente *et al.*, 1985a). The *Hind*–*Taq*5′ probe derives from the *Hind*–*Hind* insert after digestion with *Taq*I. It contains the first 228 nucleotides of the cDNA segment inserted in pAGP2. Densitometric analysis was done using a Bio-rad videodensitometer (model 620).

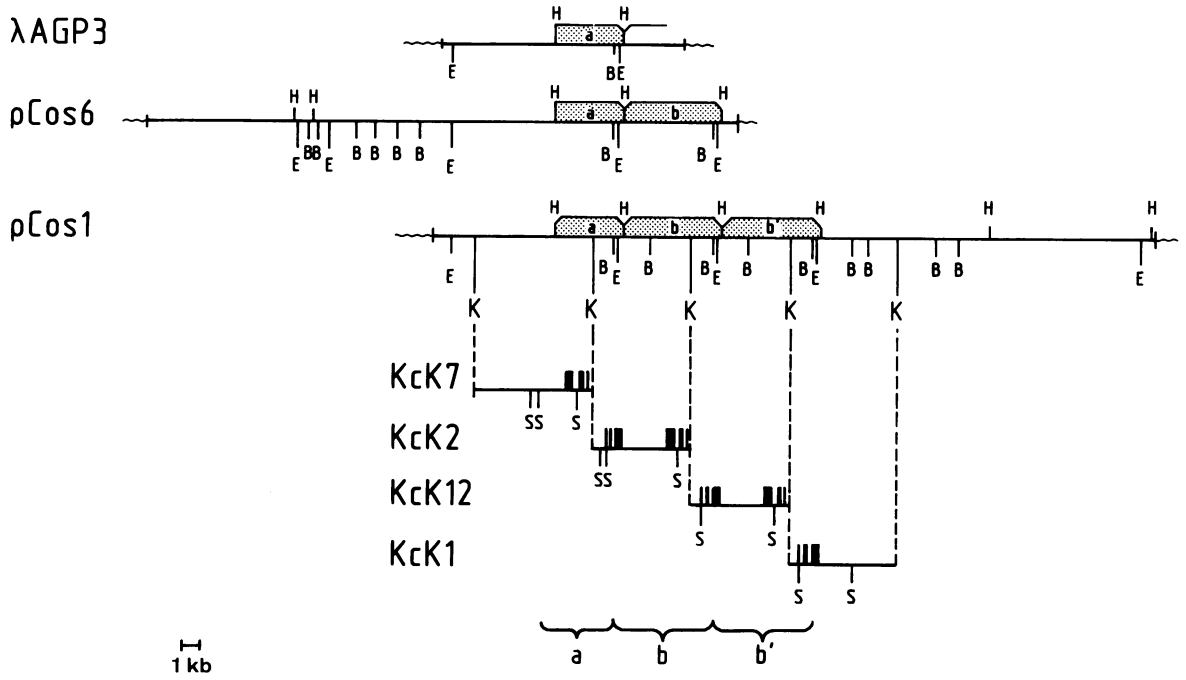

Fig. 2. Restriction map of α1-acid glycoprotein genomic clones and subclones. Shaded boxes represent AGP-A, AGP-B, AGP-B′ genes (indicates as a, b and b′). λAGP3 is the previously characterized λ clone: pCos1 and pCos6 derive from a cosmid library in pCos2EMBL. Subclones of pCos1 in pUC19 are indicated as follows: KcK7 contains the 5′ flanking region and the first three exons of the gene A. KcK2 contains the last three exons of gene A and the first three exons of gene B. KcK12: the last three exons of gene B and the first three of gene B′. KcK1: the last three exons of gene B′. KcK2 and KcK12 have the same size but can be distinguished by the presence of an *Sph*I site in KcK2 and not in KcK12. Thick bars are exons. H, *Hin*dIII; B, *Bam*HI; E, *Eco*RI; S, *Sph*I; K, *Kpn*I.

proteins (Dente *et al.*, 1985a). In this paper we show that there are three AGP coding regions in the human genome, which we call AGP-A, AGP-B and AGP-B′. They are clustered in one locus and their sequence accounts for almost all the alternative amino acids found by Schmid (1975). We present results on their relative expression and regulation.

Results

Isolation of AGP genomic clones

We have previously described a human genomic segment containing a complete AGP gene whose exonic sequence was identical to that of several AGP cDNAs (Dente *et al.*, 1985a). Restriction mapping showed that the entire coding region was included within a *Hin*dIII fragment 4.6 kb long. Southern blot analysis of *Hin*dIII-digested human DNA, using as probe nick-translated AGP-cDNA, reveals the 4.6-kb segment but also an additional 6.5-kb band (Figure 1). We have found the same two bands in every individual examined so far, but their relative amount was different in different individuals (Dente *et al.*, 1985a and unpublished work). We decided to clone the genomic segment corresponding to the 6.5-kb band. For this purpose we screened a cosmid library derived from DNA extracted from the peripheral blood of the individual (C.S.) analysed in Figure 1. In this case, the relative amounts of the 6.5- and the 4.6-kb band appeared to be in the ratio of 49:40 when total AGP cDNA was used as probe, and of 46:26 when a shorter DNA segment was used, deriving from the 5′ half of the AGP cDNAs. This difference is due to differences in the AGP coding sequences which, as we found out later, are clustered in the 3′ half of the AGP coding region. The ratio of 46:26 is the correct one (see later) and therefore we expected to find two copies of the 6.5-kb segment per copy of the 4.6-kb segment.

Screening of a cosmid library (Poustka *et al.*, 1984) yielded several positive clones which were further analysed. Restriction enzyme mapping of the cosmids showed that all clones carried inserts containing overlapping segments, covering ~70 kb of the human genome. Within this region we identified three adjacent AGP genes, whose organization is diagrammatically shown in the upper part of Figure 2, where the restriction map of two cosmids is reported in detail, along with the restriction map of the λ-AGP clone described previously (Dente *et al.*, 1985a).

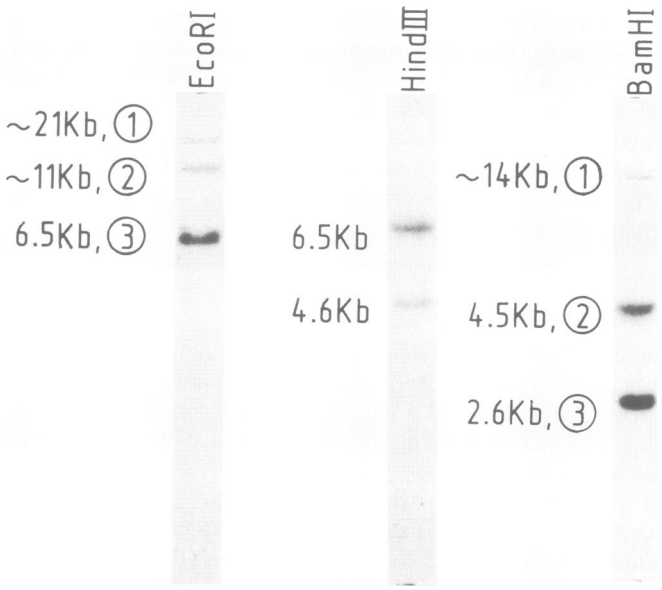

Fig. 3. Southern blot analysis of human DNA restricted with *Eco*RI, *Hin*dIII and *Bam*HI. The probe used was the *Hin*dIII–*Hin*dIII insert of plasmid pAGP2.

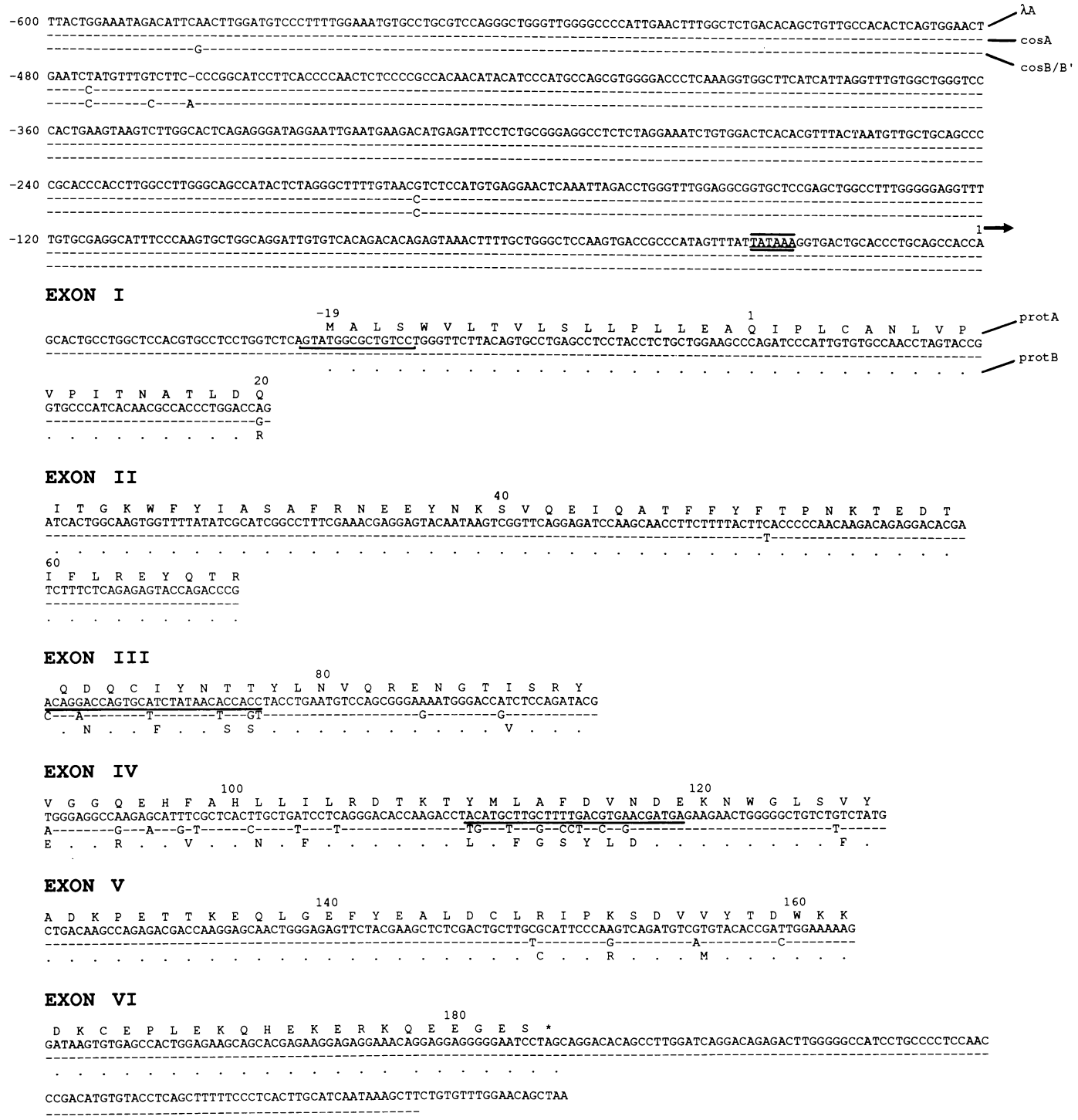

Fig. 4. Sequence of the AGP genes. DNA sequences of the 5′ flanking and coding regions of gene AGP-A isolated from λAGP3, gene AGP-A from pCos1, genes B/B′ from pCos1 and their deduced amino acid sequences. Dashed lines represent regions identical to the reported sequences. Amino acids are numbered as in the protein sequence of Schmid *et al.* (1975). The major start site of transcription is indicated with an arrow. The putative TATA sequence is boxed. The regions from which specific oligonucleotides (opposite strand) are derived are underlined.

Regions coding for AGP were localized by Southern blot analysis (data not shown). In pCos1 three AGP coding sequences are clustered: indicated as a, b and b′. AGP-A is included in a 4.6-kb *Hin*dIII segment, and clearly corresponds to the AGP gene identified and sequenced previously (Dente *et al.*, 1985a). Downstream to this there are two adjacent 6.5-kb *Hin*dIII segments, defining two apparently identical genes (B and B′) which differ from A by the absence of an *Sph*I site.

The gene cluster contains all the AGP sequences present in the human genome

We addressed the question of whether the three clustered AGP sequences present in the characterized cosmids represent all human AGP genomic sequences. Seven other cosmids were analysed by restriction mapping and were shown to derive from different cloning events but from the same genomic segment as

Table I.

Position	Schmid	gene A	gene B	Exon
20	Q,R	Q	R	I
32	F,A	F	F	II
47	T,A	T	T	II
70	D	D	N	III
73	I,F	I	F	III
76	T,S	T	S	III
77	T,S	T	S	III
88	I,V	I	V	III
92	V,E	V	E	IV
95	Q,R	Q	R	IV
98	F,V	F	V	IV
101	L	L	N	IV
103	I	I	F	IV
110	Y,L	Y	L	IV
112	L,F	L	F	IV
113	A,G	A	G	IV
114	F,S	F	S	IV
115	D,Y	D	Y	IV
116	V,L	V	L	IV
117	N,D	N	D	IV
126	V,F	V	F	IV
149	R,C	R	C	V
152	K,R	K	R	V
156	V,M	V	M	V

the pCos1 described in the previous section. We then analysed this region of the human genome by Southern blot with various enzymes: the bands obtained with *Eco*RI, *Hin*dIII and *Bam*HI are shown in Figure 3. The size and the intensity of these bands corresponds precisely to those expected on the basis of the cosmids' restriction map. *Eco*RI, bands 1, 2 and 3 correspond to the 3′ flanking region of the cluster, the 5′ flanking region and gene A and duplicated segments encoding B and B′ respectively. *Hin*dIII 6.5 and 4.6 are as explained above. *Bam*HI, band 1 corresponds to the 5′ part of the cluster whereas bands 2 and 3 correspond to the *Bam*−*Bam* segments internal to the cluster in the expected ratio of 2:3. This result shows that the cluster of genes contained in our cosmid is unique in the genome and that there are no other sequences hybridizing to AGP cDNA.

Subcloning and sequencing of AGP-A, AGP-B and AGP-B′

To sequence the exonic and flanking regions of AGP-A, AGP-B and AGP-B′, we subcloned several DNA segments from pCos1, using the strategy shown in the lower part of Figure 2. Details of the various constructs are reported in the legend to Figure 2. DNA sequencing was performed on various segments from KcKs inserts subcloned in M13 or pEMBL vectors (Messing, 1983; Dente *et al.*, 1985b). The results are reported in Figure 4. Genes AGP-B and AGP-B′ have identical exons. Gene AGP-A is different from these in several positions, indicated in Figure 4, and has a coding sequence identical to the previously sequenced gene from the λ clone. The 5′ flanking region is almost identical in all genes, the few differences are shown.

The differences in the coding sequences are interesting: they almost invariably correspond to positions which had been found to be heterogeneous in the αAGP protein sequence determined several years ago by Schmid (1975). In Table I we report the deduced amino acid sequence from AGP-A and AGP-B, and, for comparison, the alternative amino acids found by Schmid (1975), at the same positions.

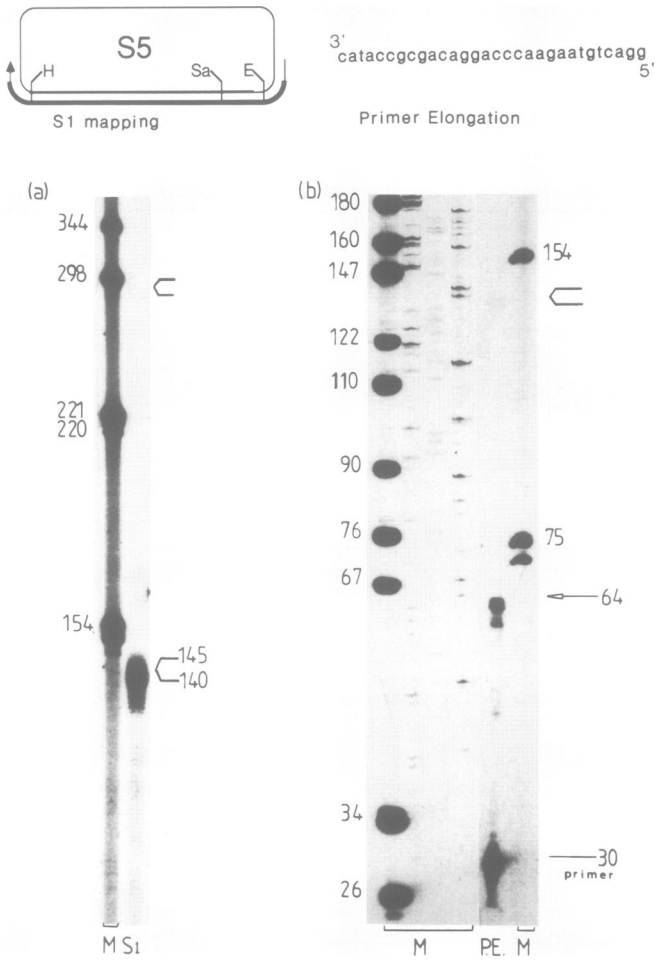

Fig. 5. Identification of the site of transcription initiation. **Panel a**: S1 mapping experiment on human liver RNA, using a uniformly labelled probe synthesized *in vitro* by primer extension on S5, a subclone of λAGP3 in mp19, extending from the *Hin*dIII site at the 5′ end of gene AGP-A to nucleotide +141 in exon I. **Panel B**: primer elongation experiment on human liver RNA using as primer the synthetic oligonucleotide shown in the top part of the figure. It corresponds to the region of exon I underlined in Figure 4. Elongation bands are indicated by arrows. Molecular weight markers (M) are *Hin*fI- and *Hpa*II-digested pBR322 DNA and a sequence ladder.

Transcription of the AGP genes

Northern analysis performed on human liver RNA indicated that AGP mRNA is ~850 bases long (Dente *et al.*, 1985a). To define more precisely the point of transcription initiation we analysed the AGP mRNA by S1 mapping and primer elongation. The results are shown in Figure 5. Both experiments are concordant with a major initiation point at an A residue 36 bases upstream from the initiator ATG codon. This result was unexpected because among the various AGP cDNAs isolated and sequenced we identified one, pAGP2 (Dente *et al.*, 1985a), which included a longer 5′ segment, up to 78 bases from the initiator ATG. Faint bands, indicated with arrows, are present further upstream; on overexposure, several minor elongation products light up also in primer elongation experiments performed using other primers (see Figures 8 and 9). It is likely, therefore, that in addition to the major start site at position −36 from the ATG, there is a minor site or sites further upstream. To confirm that the major initiation point is at −36, and that the promoter is localized im-

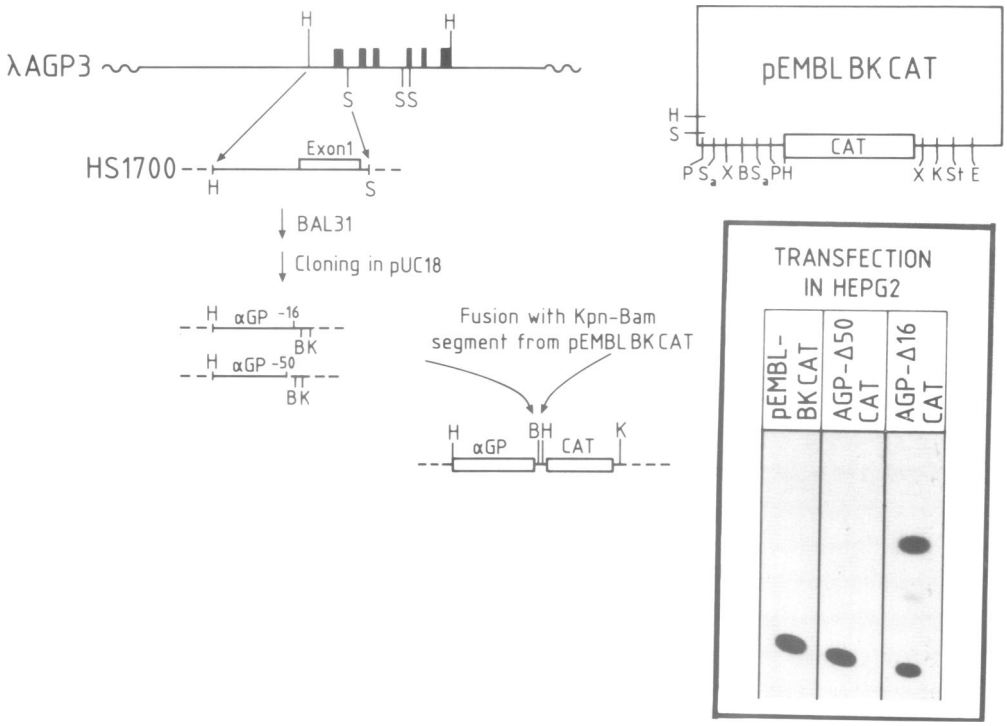

Fig. 6. Construction and expression of recombinant plasmids carrying AGP-CAT fusions. A *Hin*dIII−*Sph*I fragment containing 5′ flanking region and exon I of AGP-A was subcloned from λAGP3 into plasmid HS1700. The *Sph*I end (S) of HS1700 was treated with *Bal*31 and after digestion with *Hin*dIII the fragments were inserted in pUC18 cut with *Hin*dIII and *Hin*dII. Δ-16 and Δ-50 indicate the end-point after *Bal*31 treatment of the constructions chosen for expression studies. The coding region of the bacterial enzyme chloramphenicol acetyl transferase (CAT) was excised from plasmid pEMBLBKCAT as a *Bam*−*Kpn* fragment and inserted into the *Bam*−*Kpn*-digested *Bal*31 derivatives. Expression of these recombinant plasmids was studied by assaying the CAT enzymatic activity after transfection in HepG2 cells.

mediately upstream with the TATA box underlined in Figure 4, we have constructed a series of plasmids in which ~1250 bases from the 5′ flanking region of the AGP-A gene were fused to the CAT (chloramphenicol acetyl transferase) coding region, as shown in Figure 6. These constructs were transfected in the Hepatoma cell line Hep G2 (in which the endogenous AGP gene is efficiently transcribed) and the expression of the genes was monitored by assaying the CAT enzymatic activity. Construct AGP-Δ16cat, where the fusion was to position −16 from the start point, thus conserving the putative TATA box, was active; whereas construct AGP-Δ50cat, in which the putative TATA box has been deleted, is inactive.

Most AGP mRNA is transcribed from AGP-A

The high homology between AGP-A and AGP-B did not allow us, in the experiments reported above, to establish whether both are transcribed and in which proportion. Inspection of the sequence shown in Figure 4 indicates that it is possible to synthesize gene-specific probes: in exon 3 there is a region containing six differences over 28 bases; in exon 4 there is a region containing nine differences over 28 bases. We synthesized the oligodeoxynucleotides shown in Figure 7: ol3a and ol3b are identical to part of the third exon of gene A and B respectively; ol4a and ol4b are identical to parts of the exon 4 of gene A and B respectively (Figure 4). The experiment shown in Figure 7 convincingly shows that these oligonucleotides can be used as gene-specific probes. In this experiment, DNA from subclones of gene A, B and B′ was digested, fractionated, blotted and hybridized to the various oligos and the filter washed at various temperatures. After the 55°C wash there is absolute specificity of hybridization for all oligos, but is is clear that ol4 discriminates between gene A and B already at room temperature.

On the basis of these results, a primer elongation experiment on human liver mRNA was performed (Figure 8), using as primers ol3a and ol4a which should hybridize only to transcripts from gene A; and ol3b and ol4b which should hybridize to transcripts from B and B′. The expected bands are 320 bases long with oligo 3 and 445 with oligo 4. It is clear from these results that AGP-A is actively expressed and that the major transcriptional start point is located at position −36 from the initiation ATG codon. After overexposure, we also observe a very faint band using AGP-B specific oligos. It is possible, therefore, that AGP-B is also expressed, though at a very low level. After overexposure other minor elongation bands are detectable upstream to the major one. These minor products (see also Figure 9) have not yet been more precisely characterized. Intermediate bands are products of premature termination of reverse transcriptase.

Regulation of AGP genes in human liver and hepatoma cell lines

The results presented in the preceding sections indicate that AGP-A is actively expressed in human liver whereas AGP-B/B′ is expressed at least 100-fold less. AGP is an acute phase reactant. Its concentration in plasma increases considerably during the acute phase of infections and in other pathological states. Could AGP-B be more actively expressed during the acute phase reaction? We could not address this question directly because of the difficulty of experimentation on human patients. We therefore decided to take advantage of the observations of Darlington *et al.* (1986) who used hepatoma cell lines as an *in vitro* model system for the acute phase reaction.

Hep3B is a well-characterized human hepatoma cell line

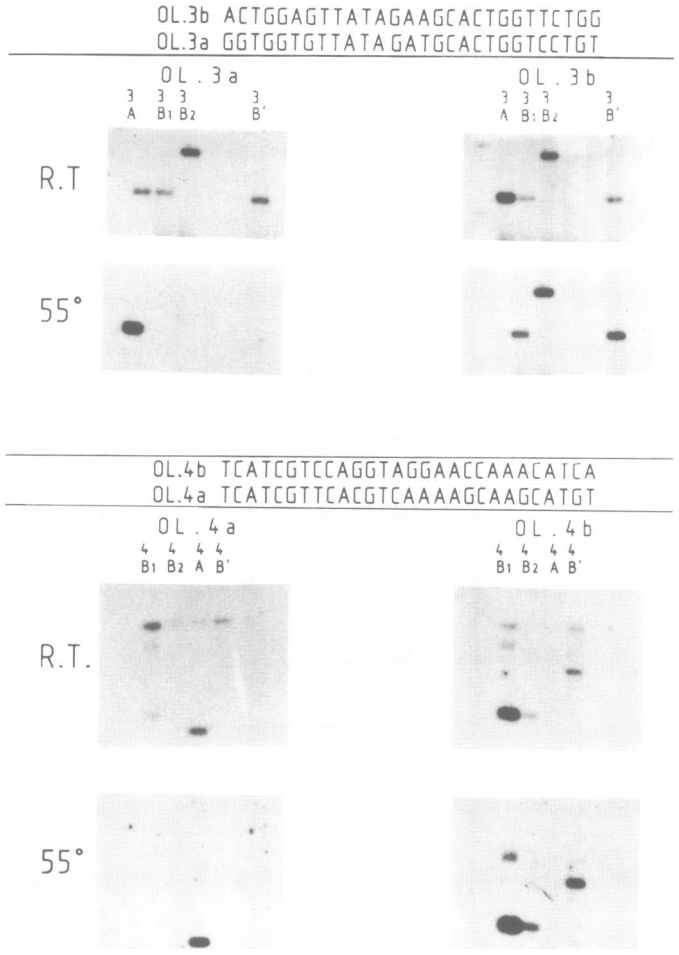

Fig. 7. Specificity of hybridization of oligonucleotides ol3A, ol3B and ol4A, ol4B. DNA from different subclones of pCos1 in mp18, was digested and transferred in duplicate on nitrocellulose filters. 3A, 3B1, 3B′, contain an 890-bp *KpnI*−*SphI* insert corresponding to exon 3 from gene AGP-A, AGP-B and AGP-B′ respectively. 3B2 contains exon 3 from AGP-B′ as a 1350 *SphI*−*SphI* insert. 4B1, 4B2 are two different preparations of DNA from the same subclone 4B, containing a 450-bp *KpnI*−*SphI* insert corresponding to gene AGP-B. 4A contains exon 4 from AGP-A as a 220-bp *SphI*−*SphI* insert. 4B′ contains exon 4 from AGP-B′ as a 1350-bp *SphI*−*SphI* insert. Oligonucleotides were labelled at the 5′ end with [γ-^{32}P]ATP, hybridized to filters at room temperature. In the figure autoradiographs are shown after a first wash at room temperature and subsequent wash at 55°C.

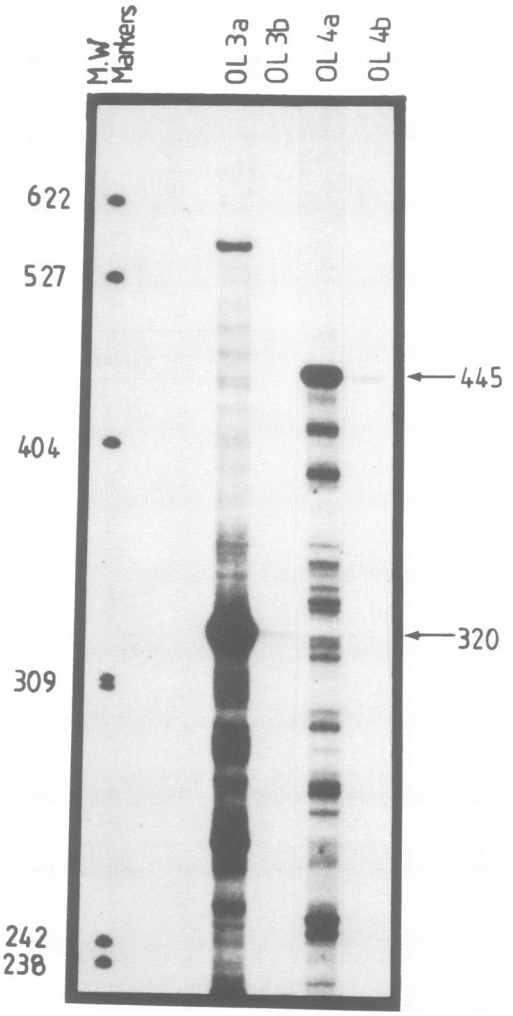

Fig. 8. Primer elongation experiment on human liver RNA using as primers ol3A, ol3B and ol4A, ol4B. Specific elongation bands are indicated by arrows. The autoradiogram was overexposed to detect elongation products deriving from ol3B and ol4B.

(Knowles *et al.*, 1980) extensively used for studies of gene expression and secretion. Recently Darlington *et al.* (1986) have shown that these cells can be 'stimulated' *in vitro* by treatment with the supernatant of LPS stimulated monocytes (SMS). We investigated the behaviour of the AGP genes in Hep3B. Northern analysis reveals that AGP mRNA is present in very low amounts in Hep3B cells; upon treatment with SMS, however, there is a strong induction of AGP mRNA. Primer extension analysis shows clearly that also in this system AGP-A is the active gene. AGP-B, is not detectably responsive to acute phase stimuli in the Hep3B model system.

Discussion

Correlation between AGP amino acid sequences and the structure of the genes

The amino acid sequence of αAGP purified from plasma pooled from many individuals is heterogenous: in certain positions (see Table I) alternative amino acids were found. Schmid (1975) speculated on the possibility that multiple sequences might be generated by a mechanism in some respects similar to that responsible for antibody diversity. Our analysis of the AGP genes offers a straightforward explanation for the heterogeneity of AGP: simply, there are two different genes coding for slightly different proteins. Inspection of the data presented in Table I, while on the whole in agreement with this explanation, shows, however, five discrepancies. For instance, not all alternative amino acids were found in one or the other gene. As shown in the Table I, both gene A and B have F32 and T47, whereas in the amino acid sequence reported by Schmid, there were alternative amino acids at positions 32 (F and A) and 47 (T and A). At positions 70, 101 and 103 there were no alternative residues, but only D, L and I respectively; we found these amino acids in gene A, but N, N and F in gene B at the corresponding positions. These differences probably reflect polymorphism in the human population.

The work of Schmid and co-workers proves that a protein very similar to the translational product of AGP-B, and in any case distinct from the protein coded by AGP-A, is present in human plasma. The fact that they found these alternative amino acids in lower yield (Emura *et al.*, 1971) is in agreement with our find-

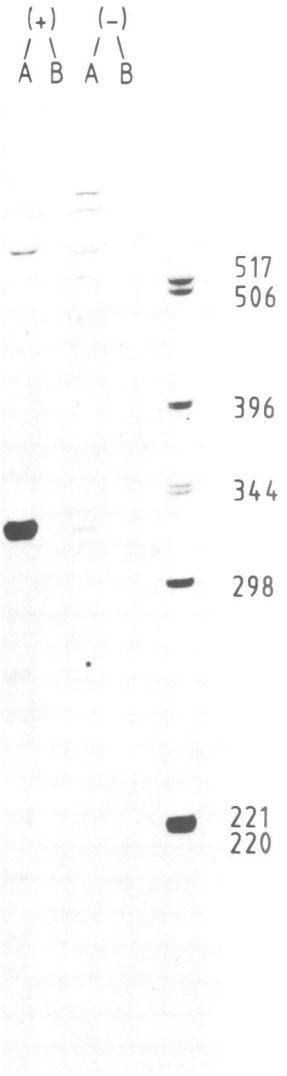

Fig. 9. Induction of AGP mRNA in the Hep3B cell line. Primer elongation experiment performed on Hep3B cells incubated for 48 hours with 10% culture medium from LPS stimulated monocytes; (+) and from untreated cells (−). A and B indicate the specific oligonucleotides used as primers: ol3A and ol3B.

ing that the AGP-B transcript was very poorly represented in each liver RNA examined.

Expression and regulation of AGP-A

S1 mapping and primer elongation experiments show that the main initiation of transcription occurs at position −36 from the initiator ATG. Minor initiation points, which have not been precisely characterized, are observed further upstream (Figures 5, 8 and 9). These might explain the finding of a cDNA clone (Dente *et al.*, 1985a) longer than the predicted major transcript. It must be stressed, however, that we found many shorter cDNAs, which might well derive from a mRNA initiating at position −36. The levels of AGP mRNAs are different in the various human livers that we examined. In all cases, however, the different amounts measured by Northern blot (data not shown) were accounted for by corresponding amounts of the −36 band detected in the primer elongation experiments, with oligonucleotides specific for AGP-A transcripts.

Darlington *et al.* (1986) have shown that Hep3B cells react to several stimuli by increasing the mRNA levels for many plasmaproteins, including AGP, in a way which resembles the acute phase response. We have performed similar experiments to examine inducibility levels in the two different genes and we observed a 10- to 20-fold increase of the level of AGP mRNA following treatment of Hep3B cells with culture medium of LPS-stimulated human monocytes. Only AGP-A is induced in this system and all the increase is accounted for by an increase in the −36 mRNA.

We do not know why AGP-B and AGP-B′ are so poorly expressed. The 5′ flanking region contains all the information to direct transcription of a downstream coding sequence, as shown by the activity of the AGP-Δ16cat construct (Figure 6); it is practically identical in all three genes. One possibility is that information present in the introns (most of which have been sequenced without revealing any interesting difference; unpublished data) might be responsible for differential expression of AGP-B. The other possibility that comes to mind is a positional effect, whereby transcription is essentially limited to the first gene in the cluster. There is no known mechanism that specifically supports this hypothesis but there are similar phenomena in other systems: for instance, human haptoglobin genes are also found in a cluster (Bensi *et al.*, 1985; Maeda, 1985), and only the first gene is efficiently transcribed (Bensi *et al.*, 1985) even though the 5′ flanking region derived from the second gene is perfectly capable of directing efficient and hepatoma-specific transcription (Raugei *et al.*, 1986). Cullen *et al.* (1984) observed a phenomenon of transcriptional interference between the 5′ and 3′ LTRs of an avian retroviral provirus. In another study, Proudfoot (1986) has shown that transcription of the first gene in a cluster interferes with the transcription of the following gene, and that the insertion of an efficient terminator sequence between the two diminishes this effect. The importance of these observations lies in the fact that in some well-defined systems (globins, α-fetoprotein-albumin) clustered genes are differentially regulated during development.

Materials and methods

Bacterial strains, vectors

Escherichia coli K12 (strain 71/18 and K514) was used for transformation (Gronenborn and Messing, 1978). Plasmids pEMBL18 and pEMBL19 (Dente *et al.*, 1985b), m13 derivatives mp18, mp19 (Messing *et al.*, 1983) and plasmids pUC18 and pUC19 (Norrander *et al.*, 1983) were used for subcloning and sequencing. The cosmid library in pCos2EMBL was a gift from A.Frischauf and was constructed as described in Poustka *et al.* (1984).

pEMBLBKCAT structure is indicated in Figure 6, details of the construction will be published elsewhere.

Enzymes and chemicals

Restriction endonucleases, T4 DNA ligase, T4 polynucleotide kinase, DNA polymerase I holoenzyme and Klenow fragment, *Bal*31 were purchased from BRL (Neu Isenburg, FRG) and New England Biolabs (Schwalbach, FRG). AMV reverse transcriptase of Seikagaku was a gift from C.Schneider. ^{32}P-labelled compounds were purchased from Amersham (Braunschweig, FRG). ^{14}C-labelled chloramphenicol was from NEN (Dreieich, FRG) and acetyl-coenzyme A from Sigma (St Louis, MO).

Oligodeoxynucleotide synthesis

Oligodeoxynucleotides were synthesized following the phosphite-amidite method (Winnacker and Dorper, 1982).

Blot hybridization and nucleotide sequence analysis

Restriction mapping of cosmids was done according to Rackwitz *et al.* (1985). Southern and Northern blot analysis was performed as previously described (Dente *et al.*, 1985a). Sequence analysis was carried out by the dideoxy-method (Sanger *et al.*, 1977).

Primer elongation and S1 mapping

Probe for S1 mapping experiment was synthesized *in vitro* as shown in Figure 5 by primer extension of single-stranded S5 subclone with m13 pentadecamer primer from Biolabs. The experiment was carried out as previously described (Ciliberto *et al.*, 1985).

Primer elongation experiments with specific oligonucleotides were carried out as described by D'Onofrio *et al.* (1985) with a few modifications to improve the specificity of hybridization: 10 μg of total RNA were annealed with 0.1 pmol of oligos at 55°C for 1 h. After isopropanol precipitation to eliminate unannealed oligonucleotides, polymerization by reverse transcriptase was performed at 42°C.

Cell culture

HepG2 and Hep3B were cultured as monolayers in Dulbecco's modified Eagle's medium 10% fetal calf serum supplemented with glutamine (5 mM final concentration) and penicillin/streptomycin (100 U/ml). Induction was obtained by incubating Hep3B cells in the presence of culture medium from LPS-stimulated monocytes as described by Darlington *et al.* (1986), except that we used 10% serum.

Transfections and CAT assays

Recombinant plasmids AGP-CAT were transfected into HepG2 cells by the calcium phosphate precipitation technique (Graham and Van der Eb, 1973) at a concentration of 10 μg/500 000 cells. Forty-eight hours after washing out the DNA–calcium precipitate, cells were harvested and CAT enzymatic activity was assayed on cellular extracts according to Gorman *et al.* (1982).

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References

Bensi,G., Raugei,G., Klefenz,H. and Cortese,R. (1985) *EMBO J.*, **4**, 119–126.
Ciliberto,G., Raugei,G., Costanzo,F., Dente,L. and Cortese,R. (1985) *Cell*, **32**, 725–733.
Cullen,B.R., Lomedico,P.T. and Ju,G. (1984) *Nature*, **307**, 241–245.
Darlington,G.J., Wilson,D.R. and Lachman,L.B. (1986) *J. Cell Biol.*, **103**, 787–793.
Dente,L., Ciliberto,G. and Cortese,R. (1985a) *Nucleic Acids Res.*, **13**, 3941–3952.
Dente,L., Sollazzo,M., Baldari,C., Cesareni,G. and Cortese,R. (1985b) In Rickwood,D. and Hames,B.D. (eds), *DNA Cloning. A Practical Approach.* IRL Press, Oxford, pp. 101–107.
D'Onofrio,C., Colantuoni,V. and Cortese,R. (1985) *EMBO J.*, **4**, 1981–1989.
Emura,J., Ikenaka,T., Collina,J.H. and Schmid,K. (1971) *J. Biol. Chem.*, **246**, 7821–7823.
Gorman,C.M., Moffat,L.F. and Howard,B.H. (1982) *Mol. Cell. Biol.*, **2**, 1044–1051.
Graham,F. and van der Eb,A. (1973) *Virology*, **32**, 456–457.
Gronenborn,B. and Messing,J. (1978) *Nature*, **299**, 797–802.
Knowles,B.B., Howe,C.C. and Aden,D.P. (1980) *Science*, **209**, 497–499.
Kuschner,I., Gewury,H. and Benson,M.D. (1981) *J. Lab. Clin. Med.*, **97**, 739–749.
Maeda,N. (1985) *J. Biol. Chem.*, **260**, 6698–6709.
Messing,J. (1983) *Methods Enzymol.*, **101**, 28–78.
Norrander,J., Kempe,T. and Messing,J. (1983) *Gene*, **26**, 101–106.
Poustka,A., Rackwitz,H.R., Frischauf,A., Hohn,B. and Lehrach,H. (1984) *Proc. Natl. Acad. Sci.*, **81**, 4129–4133.
Proudfoot,N.J. (1986) *Nature*, **322**, 562–566.
Rackwitz,H.R., Zehetner,G., Murialdo,H., Delius,H., Chai,J.H., Poustka,A., Frischauf,A. and Lehrach,H. (1985) *Gene*, **40**, 259–266.
Raugei,G., Bensi,G., Oliviero,S., Monaci,P. and Cortese,R. (1986) *Proceedings of the 34th Symposium on Proteins of the Biological Fluids*, Bruxelles, pp. 437–440.
Ritchie,D.G. and Fuller,G.M. (1983) *Ann. NY Acad. Sci.*, **408**, 490–502.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.
Schmid,K. (1975) In Putnam,F.W. (ed.), *The Plasmaproteins.* Academic Press, New York, pp. 183–288.
Winnacker,E.L. and Dorper,T. (1982) In Gassen,H.G. and Lang,A. (eds), *Chemical and Enzymatic Synthesis of Gene Fragments.* Verlag Chemie, pp. 97–102.

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