Vol. 202, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1994 Pages 65-72

CHARACTERIZATION OF THE HUMAN α_2-MACROGLOBULIN GENE PROMOTER: IDENTIFICATION OF A NOVEL, TRIPLE TRE/RARE/ERE RESPONSE ELEMENT

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Received May 16, 1994

Summary : Human α_2-macroglobulin is synthesized in the liver and in some extra-hepatic tissues but the physiological role of the protein remains unexplained. We initiated studies to characterize the promoter of the gene. In transient transfections 240 bp of the proximal promoter were necessary and sufficient for CAT-expression in HepG2 cells and lung fibroblasts. This promoter was silent in skin fibroblasts. In DNase I footprint analyses, five regions bound nuclear factors from expressing and non-expressing cells. FPII (-144 to -104) was most prominent with extracts from HepG2 cells and lung fibroblasts. In mobility shifts, FPII bound nuclear factors present in the order : HepG2 > lung >> skin fibroblasts. This region contains a canonical TRE/RARE/ERE halfsite (TGACCT) flanked by 2 related hexamers in the combinations PR4 (palindromic repeat, spacing 4) and ER1 (everted repeat, spacing 1). The interplay of (orphan) members of the steroid receptor family could explain the tissue- and species-specific regulation of the α_2M gene.

α_2-Macroglobulin (α_2M) is a wide-spectrum proteinase-inhibitor. It is considered to constitute a general or "back-up" defense mechanism against proteinases of endogenous and exogenous origin (for review, see 1). In addition, α_2M is a carrier of growth factors, cytokines and other regulatory peptides (2). In rat, α_2M is an acute phase reactant. Its expression is mainly driven by interleukin 6 (IL-6) and glucocorticoids (3-5). The promoter of the rat gene contains an IL-6 response element (6-9) but no glucocorticoid receptor binding site (10). α_2M is not an acute phase reactant in man. It is constitutively synthesized in the liver. In human hepatocytes in culture and hepatoma cells (e.g. HepG2), α_2M synthesis is only marginally stimulated by IL-6 (11). Also, the huge inhibitor (720 kDa for the tetramer) does not cross the different barriers in the body under normal conditions and α_2M is synthesized locally, e.g. by lung fibroblasts (but not by skin fibroblasts) (12,13). A specific function of α_2M in the lung is buoyed by the identification of "bait domain" mutants in COPD (chronic obstructive pulmonary disease) patients (14,15).

We have previously cloned the human α_2M gene and determined its genomic structure (14). We show here that differences in α_2M expression in HepG2 and lung versus skin fibroblasts are present at the transcriptional level: α_2M mRNA is present in HepG2 and lung fibroblasts but not in skin fibroblasts, and α_2M promoter activity is confined to

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Abbreviations : α_2M=α_2-macroglobulin; CAT=chloramphenicol acetyltransferase; ER1=everted repeat, spacing 1; IL-6=interleukin-6; GAPHD=glyceraldehyde-3-phosphate dehydrogenase; MRE=metal response element; PR4=palindromic repeat, spacing 4; PZP=pregnancy zone protein;
TRE/RARE/ERE=thyroid/retinoic acid/estrogen receptor element.

0006-291X/94 $5.00

HepG2 and l· ıg fibroblasts in transient transfection experiments. Footprinting and mobility shift experiments indicated that the region from -144 to -104 (FPII) may have a pivotal role in the expression of the α_2M gene. This region contains a potential binding site for (orphan) members of the steroid receptor family.

MATERIALS AND METHODS

Northern analysis : Northern filters with total RNA from cultured cells were hybridized with a fragment of the human α_2M cDNA (see 16). The $MTII_a$ probe used as a reference in the experiments with Zn^{2+} was generated by PCR on genomic DNA with prin ɔrs derived from published cDNA sequences (17). A GAPDH probe was used as a control.

α_2M promoter-CAT (chloramphenicol acetyltransferase) constructs and transfection experiments : The region from +12 to -4845 of the human α_2M gene was cloned into pCAT-basic (Promega, WI). The 5' deletion fragments were generated by digestion of this plasmid (pCATA2M(4845)) with *Sph*I, *Nsi*I, *Hin*dIII and *Bgl*II to generate pCATA2M(3819), pCATA2M(735), pCATA2M(231) and pCATA2M(68) respectively. HepG2 and human fetal lung and skin fibroblasts were transfected by electroporation (Genepulser, Biorad, CA) : supercoiled plasmid DNA (30 µg of pCATA2M(4845) or an equimolar amount of the other constructs) in 50 µl H_2O was mixed with 450 µl cell suspension (0.5 to 2.10^7 cells/ml). Optimal transfection efficiencies were observed at 960 µFd and 200, 280 and 300 V for HepG2, lung and skin fibroblasts respectively. Five µg of pT109-luc (18) was cotransfected for monitoring transfection efficiency. Cells were harvested after 48 hours and the CAT activities were quantified by scintillation counting after xylene extraction of butyrylated [^{14}C]-chloramphenicol (19). Luciferase activity was assayed as prescribed (Promega) and measured in a scintillation counter with the coincidence correction switched off.

DNase I footprint analysis : A 373 bp *Hin*dIII-*Dde*I restriction fragment ranging from -232 to +146 in the human α_2M gene (14) was used as a probe. To visualize the coding strand, a 585 bp *Eco*RI-*Dde*I fragment was labeled by filling both ends with the Klenow fragment of DNA polymerase I and [α-^{32}P]dCTP and digested with *Hin*dIII. For the non-coding strand, a 632 bp *Hin*dIII-*Dra*I fragment was labeled and cut with *Dde*I. The probes were gel-purified.
Nuclear extracts were prepared as described by Wegenka et al. (9), from cells grown to 80-90 % confluence in 175 cm^2 culture flasks. From 25 to 100 µg of the nuclear proteins were preincubated for 10 min on ice with 1-2 µg poly(dIdC) (Pharmacia, Sweden) in 80 µl footprinting buffer (10 mM HEPES, pH 7.9, 100 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM EDTA, 5 % glycerol, 0.05 % Nonidet-P40, 1 mM DTT and 0.05 µg/ml BSA). After addition of the probe (approximately 0.05 to 0.1 ng) the reaction mixture was further incubated on ice for another 20 min. The DNase I digestion was carried out on ice by adding 0.5 to 3 U DNase I in 20 µl of a solution containing 5 mM $CaCl_2$ in footprinting buffer. The reaction was stopped after 15 sec by a phenol-chloroform extraction. Samples were precipitated and resuspended in 5 µl loading buffer before separation on a 6 % sequencing gel.

Mobility shift assays : The sequences of the double-stranded oligomers are indicated in figure 3C. The oligomers were labeled by filling in 5' protruding ends with the Klenow fragment using [α-^{32}P]dCTP (3,000 Ci/mmol) or, for blunt-ended double-stranded oligomers, with [γ-^{32}P]ATP (6,000 Ci/mmol) and T_4-PNK (19). Nuclear extracts (0.5 to 10 µg of protein) were incubated with approximately 10,000 cpm of labeled probe in 20 µl reaction mixtures (containing 10 mM TRIS, pH 7.5, 50 mM NaCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 4 % glycerol and 1 mM DTT, with 1 µg poly(dIdC) per sample) for 15 min on ice. In experiments with the $FPII_L$ oligomer, 200 ng of sonicated and denatured salmon sperm DNA (ss DNA) was added to the mixture. In competition assays, unlabeled oligomers were added prior to the addition of the probe. Samples were electrophoresed at 4°C on a 4 % non-denaturing acrylamide gel in 0.25 x TBE (1 x TBE is 90 mM Tris-borate, 2 mM EDTA). Gels were run at 10 V/cm for 2 to 3 h, dried and autoradiographed.

RESULTS

Differential expression of the α_2M gene and functional analysis of its 5' flanking region in different cell types : The fibroblast cultures provided us with an excellent model to study the differential expression of the human α_2M gene. Northern analysis showed that the gene is transcribed in lung fibroblasts but switched off in skin fibroblasts. It is strongly expressed in HepG2 cells (figure 1A).

pCATA2M(4845) expressed CAT activity in HepG2 cells and lung fibroblasts at respectively 42 and 29% of a control in which the reporter gene is driven by an SV40 promoter and enhancer (pCAT-control). When the length of the α_2M promoter was reduced to 3819, 735 or 231 bp, the expression in HepG2 varied from 85±9 to 165±16 and 102±8% (n=3) compared to the entire construct. Promoter activity was lost when the construct was truncated at -68 bp. In lung

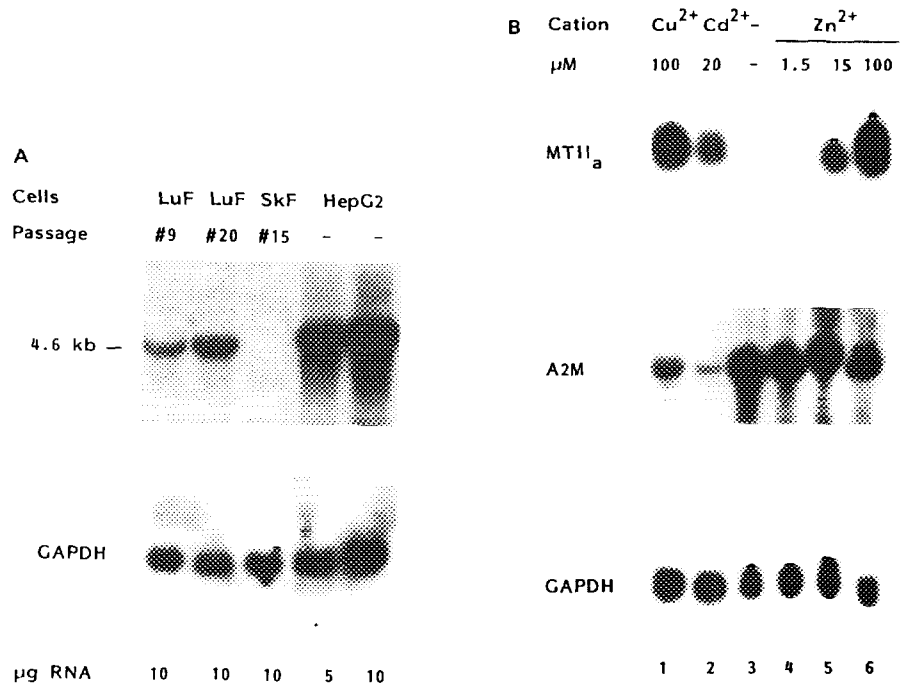

Figure 1. Northern analysis of **A.** α_2M transcription in HepG2 cells and fibroblasts in culture, and **B.** the effect of Zn^{2+}, Cu^{2+} and Cd^{2+} on α_2M expression in HepG2 cells. In **A.** total RNA from lung (LuF) and skin (SkF) fibroblasts and from HepG2 cells was loaded as indicated. In **B.** lanes contain 30 µg RNA from HepG2 cells cultured for 48 h in normal medium (lane 3) and in medium containing Cu^{2+} (100 µM, lane 1), Cd^{2+} (20 µM, lane 2) and Zn^{2+} (1.5 to 100 µM, lanes 4-6). The autoradiograms correspond to hybridizations with probes for α_2M and GAPDH, and $MTII_a$, α_2M and GAPDH respectively.

fibroblasts the activity of pCATA2M(231) was not significantly different from that of pCATA2M(4845) (29±4 versus 24±2%), just like in HepG2. Skin fibroblasts did not express any CAT activity from these constructs.

Lack of transcriptional activation by Zn^{2+} through a putative Metal Response Element (MRE) : Based upon sequence homologies, we have previously localized a putative MRE in the 5' flanking region of human α_2M (14). Because α_2M is a known carrier of Zn^{2+} in the plasma (20), one could speculate about a role of Zn^{2+} in expression of α_2M e.g. in the context of wound healing. We have tested whether α_2M expression was modulated by Zn^{2+}. HepG2 cells were treated with 1.5, 15 and 100 µM $ZnSO_4$ in the absence of fetal calf serum. After 48 h, RNA was prepared from these cultures. There was no induction of α_2M expression by Zn^{2+} under conditions that clearly induced the transcription of the human $MTII_a$ gene (figure 1B). Cu^{2+} (100 µM) and Cd^{2+} (20 µM) treatment of the cells also induced $MTII_a$ expression but not α_2M. We cannot explain the observed decrease in α_2M mRNA in response to Cd^{2+} and Cu^{2+}.

The 5'-flanking region of the human α_2M gene binds nuclear factors from both expressing and non-expressing cells : DNase I footprint analysis was chosen to localize sequences that bind nuclear factors present in HepG2 and/or lung fibroblasts but not in skin fibroblasts. Five footprinted areas, named FPI to FPV in a 5'-3' direction, were detectable in the region from -232 to +146 of the α_2M gene (figure 2).

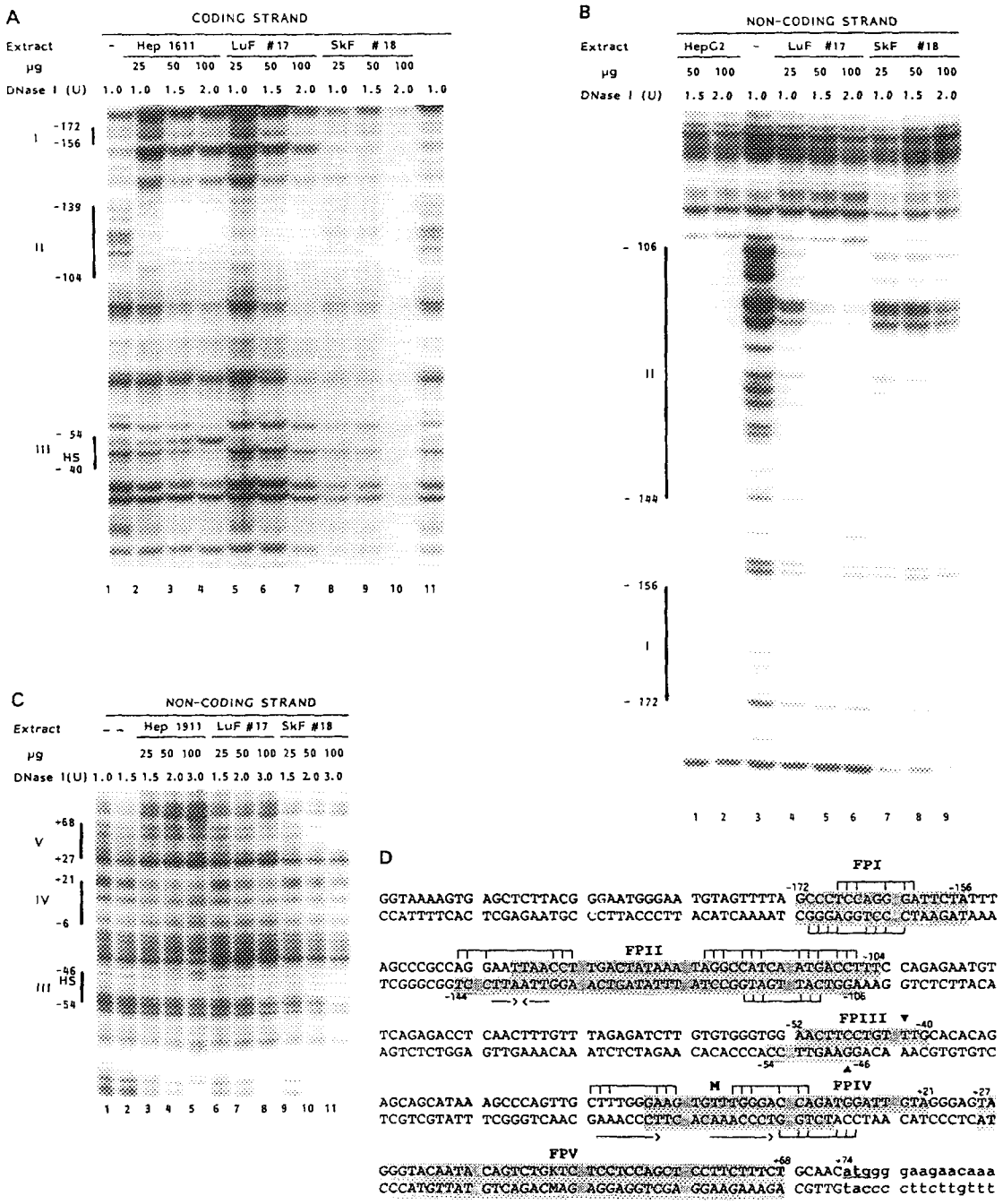

Figure 2. Footprint analysis of the human α_2M promoter. A fragment of the 5' region of the α_2M gene (-232 to +146) was end-labeled to either visualize its coding (A) or non-coding strand (B)(C). The probe was digested with DNase I (the units (U) per reaction are indicated above each lane) in the absence or presence of the indicated amounts of nuclear extracts from the different cell types (indicates the passage number at which the fibroblast cultures were harvested) (HS= hypersensitive site). In (D) a compilation of the observed footprints (shaded sequences) is given (see text for details on cell-type specific DNase I protection). M stands for major transcription initiation site at +1, and the translation start site is indicated (atg). Bars represent palindromic sequences within the footprinted regions, arrows show repeated sequences, and the hypersensitive sites within FPIII are indicated by arrowheads.

FPI, from -172 to -156 on both strands, is present with extracts from all three cell types and is presumably not responsible for the differential expression (figure 2A,B). When the promoter regions of the human and the rat α_2M gene are aligned, FPI coincides with the footprint II region observed with rat liver extracts (6). This region binds IL6-RE-BP (10) or APRF (9), nuclear factors that mediate the acute phase response in the rat. In man, two APRE-like elements in the region -219 to -182 (-248 to -211 according to (9)) in the human α_2M promoter are the homologs of the two APRE-sites within -215 to -165 in the rat. This region is exempt of footprints in our experiments. FPI might bind the constitutive CTGGAAA factor observed by Wegenka et al. (9) in mobility shift assays with extracts from HepG2.

FPII covers a large region from -139 to -104 on the coding strand, and from -144 to -106 on the non-coding strand. The region -122 to -114 within this footprint is fully protected with extracts from HepG2 and protected in a concentration-dependent way with extracts from lung fibroblasts. This protection is very weak with extracts from skin fibroblasts, even at high concentrations (figure 2A,B). Closer inspection of FPII reveals that the band at -105 on the non-coding strand is attenuated with skin fibroblast extracts as compared to HepG2 and lung fibroblast extracts and to the control digestion. The 3' end of FPII extends thus downstream of -106 in skin fibroblasts; the exact border cannot be derived from these experiments because the region -104 to -99 (non-coding strand) is not susceptible to DNase I digestion. FPII is not seen with a commercial HeLa extract (not shown). FPII was further explored in mobility shift assays (see below).

FPIII is a weak footprint with extracts from HepG2 (-52 to -40 (coding) and -54 to -46 (non-coding)) but shows an strong hypersensitive site around -42 and -45 on the respective strands. Nor the footprint neither the hypersensitive site were seen with extracts from fibroblasts (figure 2A,C). The nuclear factor involved in this footprint may thus have a role in the strong expression in HepG2. FPIII contains at its 3' end the TGTTTGC motif which is a potential target for C/EBP and EBP40/EBP45 and is important for the hepatocyte-specific expression of genes (see 21).

FPIV is a weak footprint (figure 2C) from -6 to +21, which covers the major transcription initiation site at +1 and is most obvious with extracts from HepG2. It probably reflects the binding *in vitro* of basal transcription factors.

FPV (approximately from +27 to +68) is located immediately upstream of the translation start site (+74) and, surprisingly, is more pronounced with extracts from skin fibroblasts and absent or very weak with extracts from HepG2 and lung fibroblasts (figure 2C). The footprinted region shows homology at -59 to -51 to an initiator site (22).

The FPII region binds at least one factor which is enriched in HepG2 and lung fibroblasts : Using $FPII_L$, a double-stranded oligomer which covers the whole region of FPII, two retarded complexes are observed which are specifically competed by cold $FPII_L$ (figure 3A,B). The same bands are seen with extracts from lung and skin fibroblasts, but the concentration of the nuclear factors that bind to the oligomer is different : HepG2 > lung fibroblasts >> skin fibroblasts (figure 3A). The band with the highest mobility (lower band) is also competed by oligomer $FPII_C$ (figure 3B), containing the sequence -122 to -100 (see figure 3C), but not by $FPII_A$ (-147 to -120) and $FPII_B$ (-126 to -112). None of the oligomers tested in this assay affect the upper band observed with $FPII_L$. When $FPII_C$ was labeled and used as a probe, it specifically retarded nuclear factors in a smeared band (figure 3D), which is probably equivalent to the lower band seen with $FPII_L$. The non-specific band is unaffected by cold $FPII_C$ but is competed by high concentrations of an NFY oligomer (figure 3D). Again, the intensity of the specific band is different for extracts from HepG2 and fibroblasts. $FPII_C$ encompasses the region from -122 to -114 in the α_2M promoter that shows the most striking differential protection from DNase I between HepG2 and lung and skin fibroblasts (in figure 2B). In the presence of an excess of unlabeled $FPII_C$, the protection from DNase I at -122 to -106 vanished (not shown).

An estimate of the molecular weight of the protein(s) in the bands retarded by $FPII_L$ was made by the method of Gray et al. (23). The lower band contains a protein of about 48 kDa which binds as a dimer. The approximate molecular weight was determined by subtracting 20 kDa from the apparent weight of the complex in this gel system (see 23).

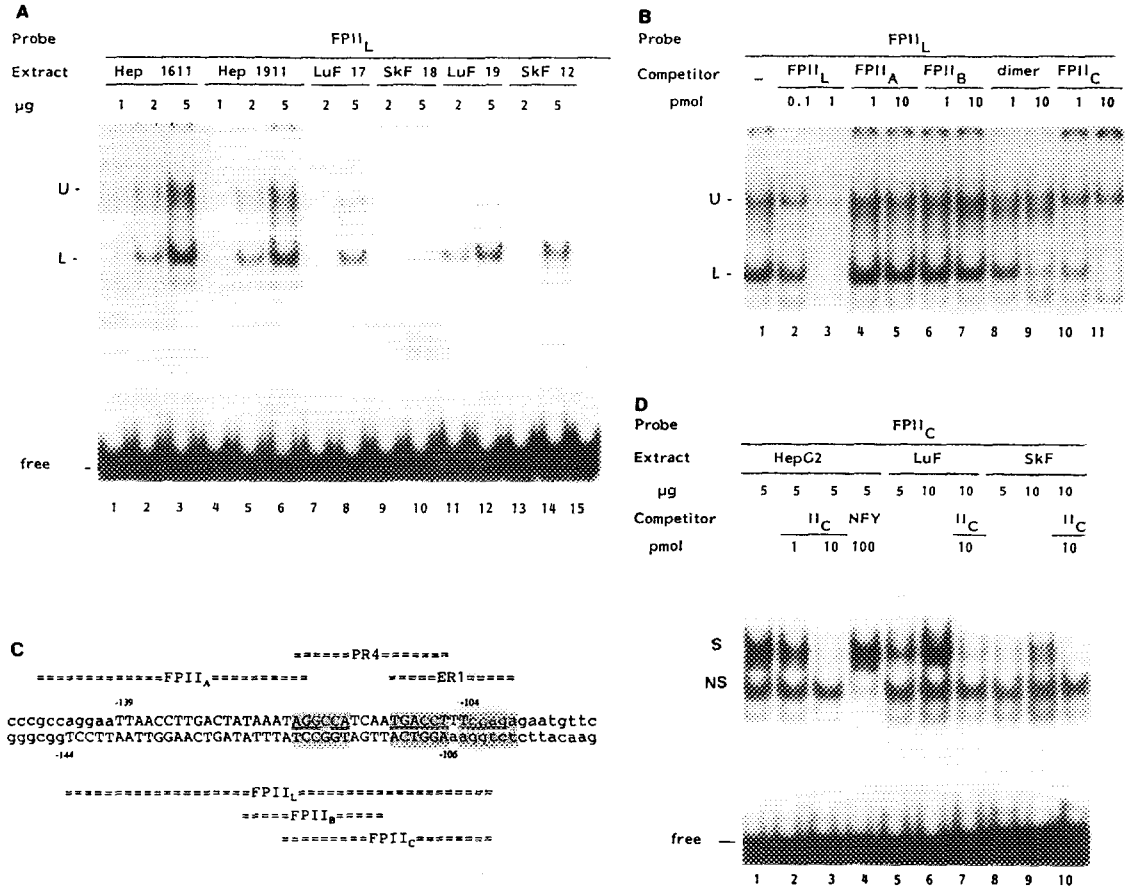

Figure 3. Nuclear factor(s) binding to FPII oligomers in mobility shift assays. In (A) the gel retardation pattern obtained with the full-length FPII$_L$ oligomer is compared for different nuclear extracts of different cell types. The free probe and the retarded upper (U) and lower (L) complex are shown. (B) represents competition experiments with cold oligomers derived from FPII. The complexes formed with 5 µg of HepG2 extract (see lane 1) are specifically competed by 0.1 to 1 pmol cold FPII$_L$ (lanes 2-3), but not by cold FPII$_A$ (lanes 4-5) or FPII$_B$ (lanes 6-7) oligomers. Dimers of FPII$_B$ compete weakly for FPII$_L$ binding (lanes 8-9). The FPII$_C$ oligomer competes specifically for the lower complex (lanes 10-11). In (C) the sequence of the FPII (-139 to -104 on the coding, and -144 to -106 on the non-coding strand) is given and the regions covered by the different oligomers are indicated. The potential TRE/RARE/ERE halfsites are boxed and the bases that correspond to the TGACCT consensus are underscored. (D) shows the retardation pattern obtained with labeled FPII$_C$ with extracts from HepG2 (lanes 1-4), lung (lanes 5-7) and skin fibroblasts (lanes 8-10). Cold FPII$_C$ oligomer (lanes 2,3,7 and 10) and an excess of cold NFY oligomer (lane 4) are used in competition assays (S, specific complex; NS, non-specific band).

DISCUSSION

The α_2M promoter does not show an apparent TATA sequence and is not GC rich, and is thus not expected to be constitutively active but rather to be prone to regulation during development or in adult life (22). As confirmed by Northern analysis, the gene is indeed expressed in a cell-type specific manner. Transient transfection experiments showed that the α_2M promoter is a strong promoter in HepG2 and lung fibroblasts with an activity that is roughly one third of that of an SV40 early promoter. The promoter is silent in skin fibroblasts. Reduction of the promoter down to the proximal 240 bp hardly affects the expression level in HepG2 and lung fibroblasts.

The footprint pattern suggests that FPII has the major role in the differential expression of the gene : expression of α_2M relates to the strength and appearance of (part of) this footprint within the α_2M promoter. Nuclear factors responsible for footprints at several other sites in the α_2M promoter probably serve to tune up the expression of the gene (see Results). The putative MRE and potential HNF-1-binding (HP-1) and Sp1 sites in the 5' flanking region of human α_2M (14) have been excluded from activity, at least *in vitro*, in different assays. First, the MRE is apparently not functional in HepG2 cells. Secondly, the potential Sp1 sites are not protected in DNase I footprint assays, even though Sp1 is present in all extracts (not shown). Finally, HNF-1 is excluded based on footprinting results : the observed footprints are lost upon heat treatment of the nuclear extracts, whereas HNF-1 is known to resist to this inactivation (24).

On the other hand, we identified a consensus TRE/RARE/ERE halfsite at -111 to -106 in FPII. In the context of steroid receptor response elements (see 25), -121 to -99 contains both a palindromic repeat spaced by 4 nucleotides (AGGcCAtcaaTGACCT, PR4) and an everted repeat with a single space (TGACCTtTCCAGa, ER1) (see figure 3C). Although the above combination of halfsites has not been found in other gene promoters, triple hexamer repeats have recently been identified in the human medium chain acyl-coenzyme A dehydrogenase gene (26), in the rat hydratase-dehydrogenase gene (27,28), the apoAI-RARE (29), and the myosin heavy chain and growth hormone gene TREs (30). It is not established yet whether the fourth, upstream halfsite (TaACCT at -138 to -133) is part of the response element in the α_2M gene promoter. The molecular weight of the factor(s) that bind $FPII_L$ was estimated at about 48 kDa, which is compatible with the size of several (orphan) steroid receptors. Electrophoresis of the retarded complex under denaturing conditions indicated that proteins bound as dimers (not shown). The detailed analysis of this novel binding site will be the subject of further investigations.

This unique response element may play a role in the cell-specific, temporal, developmental and species-specific regulation of the α_2M gene. First, different members of the steroid receptor superfamily are known to be selectively enriched in tissues or cells. Moreover, the complex nature of the response element suggests that different members of this superfamily may be involved in α_2M regulation. Second, the region might be involved in the developmental regulation of α_2M, since several steroid receptors play an important role in development. Third, we hypothesize that the response element may also have a role in the expression of the α_2M gene in the rat : the region of FPII in the human α_2M promoter is homologous between the rat and the human promoter (figure 4). Finally, the promoter regions of human α_2M and pregnancy zone protein (PZP) are homologous except for this region (figure 4). PZP is another α-macroglobulin, thus far only identified in some primates, which is only expressed during pregnancy (see 1).

The further elucidation of the transcription factors that bind to FPII may thus provide some clues to the physiological regulation of α_2M.

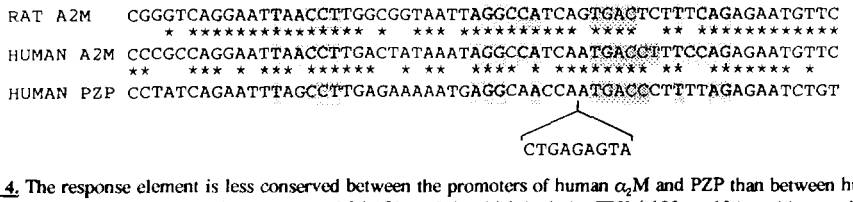

Figure 4. The response element is less conserved between the promoters of human α_2M and PZP than between human and rat α_2M. The 5'-3' sequence of the human α_2M (-150 to -91), which includes FPII (-139 to -104 on this strand) and the triple hexamer repeat (-122 to -99) is compared to the corresponding sequences of rat α_2M (-151 to -92 (28)) and human PZP (-159 to -91). Bases within the potential TRE/RARE/ERE halfsites that correspond to the consensus TGACCT are boxed. A 9-bp region in the human PZP sequence (bracketed) with no TGACCT similarities has no equivalent in the other sequences.

ACKNOWLEDGMENTS

We thank Marleen Willems, Lut Mekers and Greta Vandereycken for tissue culture and Karel Rondou for the photographic work. Peter Marynen is an "Onderzoeksleider" and Gert Matthijs a "Postdoctoraal Onderzoeker" of the "Nationaal Fonds voor Wetenschappelijk Onderzoek", Belgium.
This investigation was supported by grants 3.0025.92 and 3.0113.94 of the Fonds voor Geneeskundig Wetenschappelijk Onderzoek, Belgium; by the Inter-University Network for Fundamental Research sponsored by the Belgian Government (1991-1996) and by a grant "Geconcerteerde Acties" from the Belgian Government.

REFERENCES

1. Sottrup-Jensen, L. (1987) *In* The plasma proteins, structure, function, and genetic control (F.W. Putnam, Ed.), pp. 191-291. Academic Press, New York.
2. Gonias, S.L. (1992) Exp. Hematol. 20, 302-311.
3. Andus, T., Geiger, T., Klapproth, J., Kunz, D., Heisig, M., Castell, J., and Heinrich, P.C. (1988) Tokai J. Exp. Clin. Med. 13, 265-275.
4. Gehring, M.R., Shiels, B.R., Northemann, W., de Bruijn, M.H.L., Kan, C.-C., Chain, A.C., Noonan, D.J., and Fey, G.H. (1987) J. Biol. Chem. 262, 446-454.
5. Kunz, D., Zimmermann, R., Heisig, M., and Heinrich, P.C. (1989) Nucleic Acids. Res. 17, 1121-1138.
6. Hattori, M., Abraham, L.J., Northemann, W., and Fey, G.H. (1990) Proc. Natl. Acad. Sci. USA. 87, 2364-2368.
7. Ito, T., Tanahashi, H., Misumi, Y., and Sakaki, Y. (1989) Nucleic Acids Res. 17, 9425-9435.
8. Tsuchiya, Y., Hatori, M., Hayashida, K., Ishibashi, H., Okubo, H., and Sakaki, Y. (1987) Gene. 57, 73-80.
9. Wegenka, U.M., Buschmann, J., Lütticken, C., Heinrich, P.C., and Horn, F. (1993) Mol. Cell. Biol. 13, 276-288.
10. Hocke, G.M., Barry, D., and Fey, G.H. (1992) Mol. Cell. Biol. 12, 2282-2294.
11. Krause, E., Wegenka, U.M., Möller, A., Horn, F., and Heinrich, P.C. (1992) Biol. Chem. Hoppe-Seyler 373, 509-515.
12. Brissenden, J.E., and Cox, D.W. (1982) Som. Cell Genet. 8, 289-305.
13. Mosher, D.F., Saksela, O., and Vaheri, A. (1977) J. Clin. Invest. 60, 1036-1045.
14. Matthijs, G., Devriendt, K., Cassiman, J.-J., Van den Berghe, H., and Marynen, P. (1992) Biochem. Biophys. Res. Commun. 184, 596-603.
15. Poller, W., Faber, J.-P., Klobeck, G., and Olek, K. (1992) Hum. Genet. 88, 313-319.
16. Devriendt, K., Zhang, J., Van Leuven, F., Van den Berghe, H., Cassiman, J.-J., and Marynen, P. (1989) Gene 81, 325-334.
17. Karin, M., and Richards, R.I. (1982) Nature. 299, 797-802.
18. Nordeen, S.K. (1988) BioTechniques 6, 454-456.
19. Ausubel F.M., Burt, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (1987) Ed. Current Protocols in Molecular Biology, Green/Wiley Interscience, New York.
20. Parisi, A.F., and Vallee, B.L. (1970) Biochemistry 9, 2421-2426.
21. Petropoulos, I., Augé-Gouillou, C., and Zakin, M.M. (1991) J. Biol. Chem. 266, 24220-24225.
22. Smale, S.T., and Baltimore, D. (1989) Cell. 57, 103-113.
23. Gray, T.A., Gumucio, D.L., and Collins, F.S. (1990) Technique 2, 147-154.
24. Lichtsteiner, S., Wuarin, J., and Schibler, U. (1987) Cell. 51, 963-973.
25. Truss, M., Chalepakis, G., Pina, B., Barattino, D., Brüggemeier, U., Kalff, M., Slater, E.P., and Beato, M. (1992). J. Steroid Biochem. Molec. Biol. 41, 241-248.
26. Raisher, B.D., Gulick, T., Zhang, Z., Strauss, A.W., Moore, D.D., and Kelly, D.P. (1992) J. Biol. Chem. 267, 20264-20269.
27. Bardot, O., Aldridge, T.C., Latruffe, N., and Green, S. (1993) Biochem. Biophys. Res. Commun. 192, 37-45.
28. Zhang, B., Marcus, S.L., Miyata, K.S., Subramani, S., Capone, J.P., and Rachubinski, R.A. (1993) J. Biol. Chem. 268, 12939-12945.
29. Rottman, J.N., Widom, R.L., Nadal-Ginard, B., Mahdavi, V., and Karathanasis, S.K. (1991) Mol. Cell. Biol. 11, 3814-3820.
30. Brent, G.A., Moore, D.D., and Larsen, P.R. (1991) Ann. Rev. Physiol. 53, 17-35.