Human Molecular Genetics, Vol. 1, No. 6 371–378

Human CC10, the homologue of rabbit uteroglobin: genomic cloning, chromosomal localization and expression in endometrial cell lines

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Received June 23, 1992; Revised and Accepted August 7, 1992 EMBL accession no. X59875

ABSTRACT

Human and rat cDNAs to Clara Cell 10 kDa protein (CC10) have been previously isolated. Comparison of the amino acid sequences showed that CC10 is homologous to rabbit uteroglobin. Here we present further evidence that human CC10 is the human counterpart of rabbit uteroglobin. We have isolated the gene and have mapped its genomic localization to chromosome 11q11-qter. Sequence analysis of the 5'-flanking region reveals that the homology between the human and the rabbit gene starts at the first exon/intron boundary and extends up to −1.4 kb. A second region of 0.74 kb from −1.77 to −2.51 kb in the human 5'-flanking gene region is homologous to rabbit sequences that include four progesterone receptor binding sites which have been implicated in progesterone regulation of rabbit uteroglobin gene expression in endometrium. Sequence alignment of this region on the nucleotide level shows that only two weak progesterone receptor binding sites are partially conserved. In addition, close inspection of the human and rabbit promoters reveals that the estrogen responsive element and two recently identified *cis* elements of the rabbit promoter located between −177 and −258 bp are also absent in the human uteroglobin promoter. Despite these differences in the 5'-flanking regions of the genes, we report that the human uteroglobin mRNA is expressed in a human cell line of endometrial origin indicating that human uteroglobin is expressed in the uterus like its rabbit homologue. Thus, it appears that human uteroglobin is not only a marker for lung Clara cells but also an endometrial differentiation marker. Human uteroglobin cDNA or antibodies to the protein may be used to characterize endometrium derived tumors.

INTRODUCTION

Clara cell 10 kDa secretory protein (CC10), also called Clara cell 17 kDa protein, is a homodimer consisting of 8.5 kDa monomers that are joined by two disulfide bonds (1). It is the predominant secreted protein of lung Clara cells which are lining the bronchiolar epithelium (2,3). The physiological role of the protein is not yet completely understood. It has been reported that CC10 specifically binds methylsulfonyl-polychlorated biphenyls (4) and inhibits phospholipase A_2 (5). In the last few years the sequences of rat (6,7) and human (8) CC10 cDNAs have been reported. These cDNAs and the derived amino acid sequences show striking homologies to rabbit uteroglobin (5,7). Nevertheless, it has been doubted that CC10 is uteroglobin (5).

Like CC10 rabbit uteroglobin is a covalently bound homodimer whose three-dimensional structure is well known (9). Uteroglobin expression in rabbits has been originally reported in the uterus during the preimplantation phase (10). More recently, the protein was also detected in oviduct (11), male genital organs (12), esophagus (13) and lung (13,14). *In vitro*, several distinct properties of uteroglobin have been described. Soon after its discovery it could be shown that the steroid hormone progesterone is specifically bound by the protein (15,16). Therefore, rabbit uteroglobin was believed to be a potential carrier or scavenger of progesterone that regulates the progesterone concentration in the endometrium (17). It has also been shown to specifically bind certain methylsulfonyl-metabolites of polychlorinated biphenyls (PCBs) with even higher affinity than progesterone (18). These xenobiotic PCB metabolites accumulate in the same lung cells that secrete uteroglobin (19). Furthermore uteroglobin has been found to inhibit phospholipase A_2 (20,21,22). The relationships of all these properties and their physiological significance is still not understood and remains largely a matter of speculation.

Expression of rabbit uteroglobin in the various tissues is regulated differentially by different steroid hormones. In the endometrium, progesterone and to a lesser extent estradiol increase transcription of the gene (23,24), whereas in epididymis and lung, testosterone and cortisol, respectively, are the active hormones that enhance expression (25,26,27). Moreover, closer examination of the expressing tissues revealed that expression is restricted to certain epithelial cells. For instance, in uterus only the glandular and luminal epithelium express uteroglobin (28). In lung, expression of the protein is restricted to Clara cells.

Genomic clones encoding rabbit uteroglobin have been isolated (29,30) and regions 5' to the gene identified that are probably responsible for its hormonal regulation (31,32,33). Binding sites for purified glucocorticoid and progesterone receptors are located at positions around −2.7 kb and −2.4 kb (32). An estrogen responsive element is present at −0.25 kb (33). These steroid

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hormone receptor binding sites correlate with DNaseI hypersensitive regions found exclusively in chromatin from endometrium of hormonally stimulated rabbits (32).

Uteroglobin had been found only in rabbits and two other members of the order Lagomorpha, namely hare and pica (34). Most antisera to rabbit uteroglobin do not cross-react with proteins of other species and the rabbit uteroglobin cDNA does not cross-hybridize to DNA/RNA from other species (our unpublished data). Recently, we showed that the rat CC10 mRNA is expressed like rabbit uteroglobin not only in lung but also in the esophagus as well as in uteri of estrogen and progesterone treated female rats (7) suggesting that rat CC10 is the rat counterpart of rabbit uteroglobin. In addition, mouse CC10 is expressed in the same cell types like the rabbit uteroglobin in transgenic mice (35).

In this paper we present by evolutionary considerations further evidence that human CC10 is the human counterpart of rabbit uteroglobin. We report the cloning of the gene and mapping of its chromosomal locus. In addition, we present the nucleotide sequence of the 5'-regulatory region and compare it to the corresponding rabbit and rat sequences. Furthermore, we show that human CC10 mRNA is not only expressed in lung Clara cells but also in an epithelial cell line of endometrial origin.

RESULTS

Comparison of human and rat CC10 to rabbit and hare uteroglobin cDNAs

Previously a human cDNA clone encoding CC10 was isolated from a human lung cDNA library (8). Alignment of this cDNA clone to the rabbit uteroglobin (29), hare uteroglobin (36) and rat (6,7) CC10 cDNAs reveals overall identities in the coding region of 75%, 77% and 67%, respectively (Fig. 1). In addition, the open reading frames of the human, rabbit and hare clones have the same length of 273 bp encoding 91 amino acids. The open reading frame of the rat cDNA is 288 bp in length, due to a 15 bp extension at the 3' end of the coding region. Although there is no cross-hybridization between the rabbit uteroglobin and human CC10 cDNA clones, probably due to the absence of a longer nucleotide stretch with 100% identity, the sequence identities strongly suggest that the human CC10 cDNA encodes the human homologue of rabbit and hare uteroglobin. This idea is further supported by side-by-side comparison of the amino acid sequences (5) and evolutionary considerations.

In the coding regions of CC10/uteroglobin the average number of substitutions per nonsynonymous and synonymous site between man and rabbit are lower than between rat and man or rat and rabbit (Table 1). A similar situation is observed for other genes (for comparison see values for the apolipoprotein E gene (37) and average values for 14 other genes (38) in Table 1). Thus, the rabbit and human uteroglobin genes are more closely related to each other than to the rat gene. This finding is in agreement with previous data showing that the divergence of lagomorphs from the main mammalian lineage occured after the divergence of rodents (38,39). If human/rat CC10 and rabbit uteroglobin were two different genes, this would imply that all three species must have a CC10 and a uteroglobin gene, whereby the CC10 genes must be more closely related to each other than to the uteroglobins and vice versa. Since this is not the case these data provide good evidence that rat and human CC10 and uteroglobin are the same gene.

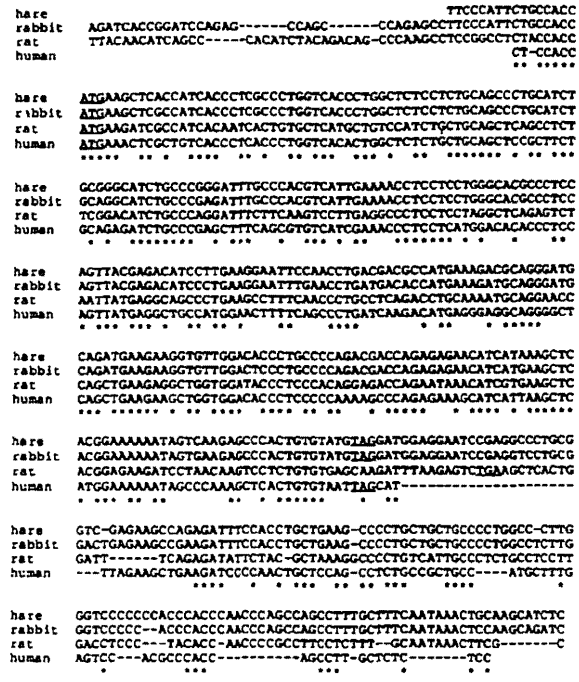

Figure 1. Alignment of hare and rabbit uteroglobin and rat and human CC10 cDNAs using the CLUSTAL program (58). The start (ATG) and the stop codons (TAG in hare, rabbit and human and TGA in rat) are underlined. Nucleotides that are identical in all four cDNA sequences are marked by asterisks. Broken lines in the 5'- and 3'-untranslated regions indicate gaps permitting best alignment.

Table 1. Average number of substitutions between genes from man, rabbit and rat. The coding regions of uteroglobin/CC10 (UG) and apolipoprotein E (Apo E) cDNAs were aligned and the number of substitutions per synonymous (above diagonal) and nonsynonymous site (below diagonal) calculated according to Li et al. (57) using the LWL90 program written by K.H. Wolfe (unpublished). For further comparison, the average number of substitutions for 14 genes taken from (38) are presented.

		Man	Rabbit	Rat
Man	UG		0.507	0.828
	Apo E		0.179	0.315
	Average		0.414	0.577
Rabbit	UG	0.250		0.811
	Apo E	0.147		0.338
	Average	0.062		0.612
Rat	UG	0.294	0.323	
	Apo E	0.213	0.248	
	Average	0.082	0.085	

Isolation of the human CC10 gene

Previously it was shown that the human CC10 gene is a single copy gene ((8) and own unpublished results) as found for the rat and rabbit genes (7,29). In order to isolate the potential regulatory 5'-flanking region of the gene we have screened approximately 500,000 independent plaques of a human genomic lambda phage library using the human CC10 cDNA as probe. A single recombinant phage was isolated and partially analyzed

by restriction enzyme mapping and Southern blotting. The insert includes approximately 20 kb of human genomic DNA. To identify and subclone a fragment that contains the 5'-flanking region of the human CC10 gene we hybridized restricted phage DNA separately with 5' and 3' parts of the cDNA. A 60 bp Eco RI-Pst I fragment from the human cDNA clone containing the codons for the 16 most NH_2-terminal amino acids hybridizes exclusively to a 4.4 kb Bam HI-Hind III fragment of the recombinant phage DNA. The remaining 3'-part (300 bp) of the human cDNA gives no signal with this DNA fragment. These results indicated that the fragment contains probably the first exon and 5'-flanking sequences. For sequencing, the Bam HI-Hind III fragment was subcloned into the polylinker site of the plasmid pSPT19. A partial restriction map of the subcloned Bam HI-Hind III fragment is shown in Fig. 2A. The 5' end of the first exon has been determined by S1 nuclease mapping with RNA from human lung. RNA from a cell line that does not express human CC10 was used as negative control. A 283 bp Pst I-Sac I fragment (see Fig. 2A) overlapping the presumed transcription start site was used as hybridization probe. The autoradiogram of Fig. 2B shows two major bands separated by two nucleotides located about 60 nucleotides upstream of the translation start point. This result suggests two different transcriptional start points separated by two nucleotides. For the assignment of these two start points to the A's at the indicated positions in Fig. 2B we took into account the different electrophoretic mobility of fragments generated by S1 nuclease and by the corresponding sequencing ladder (40). We arbitrarily assigned the proximal transcriptional start point as +1. A typical TATA motif is found thirty nucleotides further upstream. The 3' end of the first exon could be defined by the divergence of the cDNA and genomic sequences. The deduced exon/intron boundary is in agreement with the consensus donor splice site found in many other eucaryotic genes. In addition, this exon/intron boundary is found exactly at the same position in the rabbit uteroglobin and rat CC10 genes (7,30). Alignment of the human CC10 gene 5'-flanking sequence with itself using the dot matrix program of the Sequence Analysis Software Package from the Genetics Computer Group of Wisconsin (UWGCG) (41) identifies three regions that show considerable homology to each other (data not shown). These sequences are located around −3,000, −2,600 and −1,500 bp. A computer search for homologies to sequences stored in the EMBL database identifies these sequences as Alu repetetive sequences (42,43).

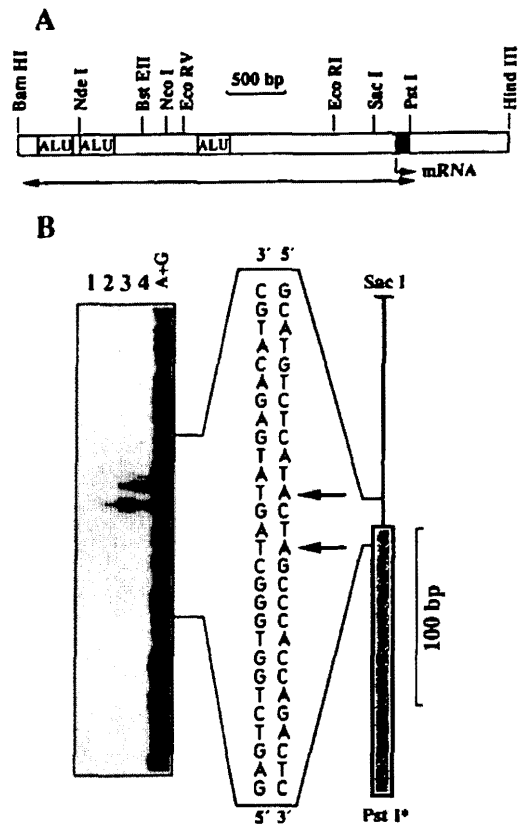

Figure 2. Structure of the human CC10 gene 5'-flanking region. A, Partial restriction map of the Bam HI-Hind III fragment obtained from the recombinant phage containing human CC10 coding sequences. The transcriptional start point is indicated by an arrow (mRNA). The shaded region denotes the first exon. The positions of the Alu sequences are marked (Alu). The double-headed arrow indicates the sequenced region. B, S1 nuclease mapping. The diagram on the right depicts the Pst I-Sac I restriction fragment around the first exon (shaded region). On the left, the autoradiogram of a 6% sequencing gel is shown. The 5' ^{32}P-labelled fragment was hybridized to 10 μg total RNA from a non-expressing cell line (lane 1) and lung (lanes 2–4), respectively. S1 nuclease digestion was performed under the following conditions. Lanes 1 and 3, 400 U S1 nuclease, lane 2, 900 U S1 nuclease and lane 4, 150 U S1 nuclease. Lane A+G, sequencing ladder. The arrows indicate the transcriptional start points.

The 5'-flanking region of the human CC10 gene is partially homologous to the rat CC10 and rabbit uteroglobin gene

Comparison of the 3.35 kb 5'-flanking sequence of the human CC10 gene with the known 5'-flanking sequences of the rabbit and rat homologues reveals two regions with considerable homology (Fig. 3). The homology to the rabbit gene starts at the first exon/intron boundary and extends up to the first Alu repeat at around −1,420 bp. It appears that the absence of homologous rabbit sequences in the human gene between −1,420 and −1,770 bp is mainly due to the substitution by an Alu repeat. The second homologous region lies between the first and second Alu repetetive sequence between −1,770 and −2,510 bp relative to the transcription start point. This region is homologous to a sequence between −2,659 and −2,333 bp of the rabbit uteroglobin gene (32) which is absent in the rat gene (7). Interestingly, in the rabbit gene this region includes four progesterone receptor binding sites that have been implicated in progesterone regulation of uteroglobin gene expression in the endometrium of rabbits during early pregnancy (32). In addition, two DNase I hypersensitive sites lie in that region of the rabbit gene (32). Sequence alignment of this region on the nucleotide level reveals that the two strong progesterone receptor binding sites (A and D in Fig. 4 A) are not conserved in the human gene. The two weak binding sites of the rabbit gene (B and C in Fig. 4 A) are partially conserved in the corresponding human 5'-flanking region. In addition, despite the obvious homology of the proximal promoter region, an estrogen responsive element found at −263 to −251 bp upstream of the rabbit uteroglobin gene (33) is completely absent in the human gene (Fig. 4B). The finding that the estrogen responsive element described for the rabbit gene is not conserved in the human gene resembles the situation in the rat CC10 gene region where this element is also absent (7).

Very recently we have identified *cis*-elements in the promoter

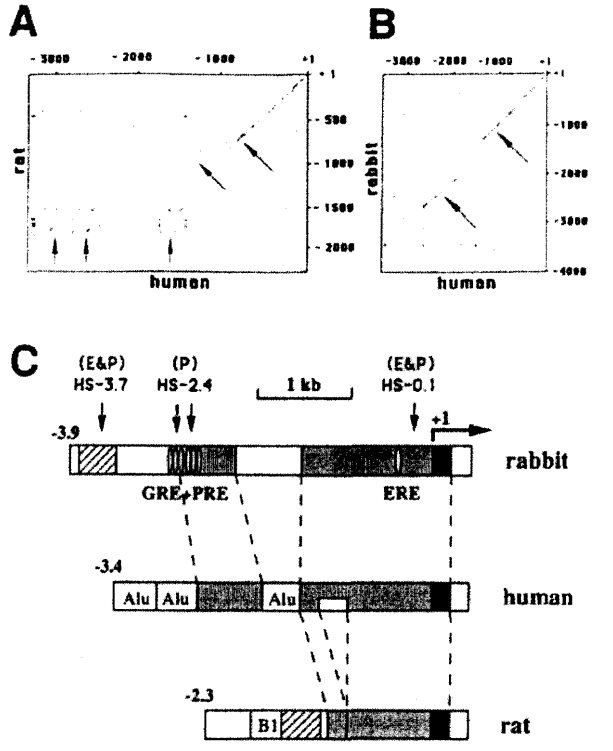

Figure 3. Similarities of the human CC10, rabbit uteroglobin and rat CC10 5'-flanking gene regions. A and B, The sequences were compared by a dot matrix program. In the matrix, each dot represents 14 identities per 21 nucleotides. Regions of homology are indicated by slanted arrows. The vertical arrows depict the Alu repetetive sequences that show some similarity to the rat B1 sequence. The human CC10 gene region from −3,349 to +1 bp is compared with the rat gene region from −2,320 to +1 bp (A) and the rabbit uteroglobin region from −3,995 to +1 (B). C, Schematic drawing of the human CC10, rabbit uteroglobin and rat CC10 5'-flanking gene regions. Hatched and shaded boxes show regions of greater than 66% overall homology. In the rabbit uteroglobin gene binding sites for glucocorticoid (GRE), progesterone (PRE) and estrogen receptor (ERE) are marked. DNaseI hypersensitive sites (HS) found in chromatin of estrogen (E) and progesterone (P) stimulated rabbit endometrium are indicated by arrows.

region of the rabbit uteroglobin gene between −400 bp and the transcription start point that mediate preferential transcription of a reporter gene in the endometrial cell line Ishikawa (44). One of these regions (−258/−220) includes a motif that is in 12 out of 13 nucleotides identical to the GT-I motif in the SV40 enhancer. This GT-I motif includes a CACCC element. Although the GT-I motif is absent in the human promoter, a CACCC motif is found between −202 and −198 bp (Fig. 4B) that is conserved at the corresponding position in the rat gene (7). Two other relevant *cis*-elements of the rabbit uteroglobin promoter, the so called uteroglobin upstream elements (UE) that are in 12 out of 13 nucleotides identical (see Fig. 4B) and bind probably the same transcription factor (44) are also not well conserved in the human promoter. In contrast, a 7 bp perfect inverted repeat (CAGTTTC) that is also found in the long terminal repeat (LTR) of all murine leukemia viruses and proviruses and therefore designated 'LTR Pal' is conserved between the rabbit und human promoter (Fig. 4B). In addition, two identical A/T rich sequence motifs that are similar to the octamer motif found in many eucaryotic

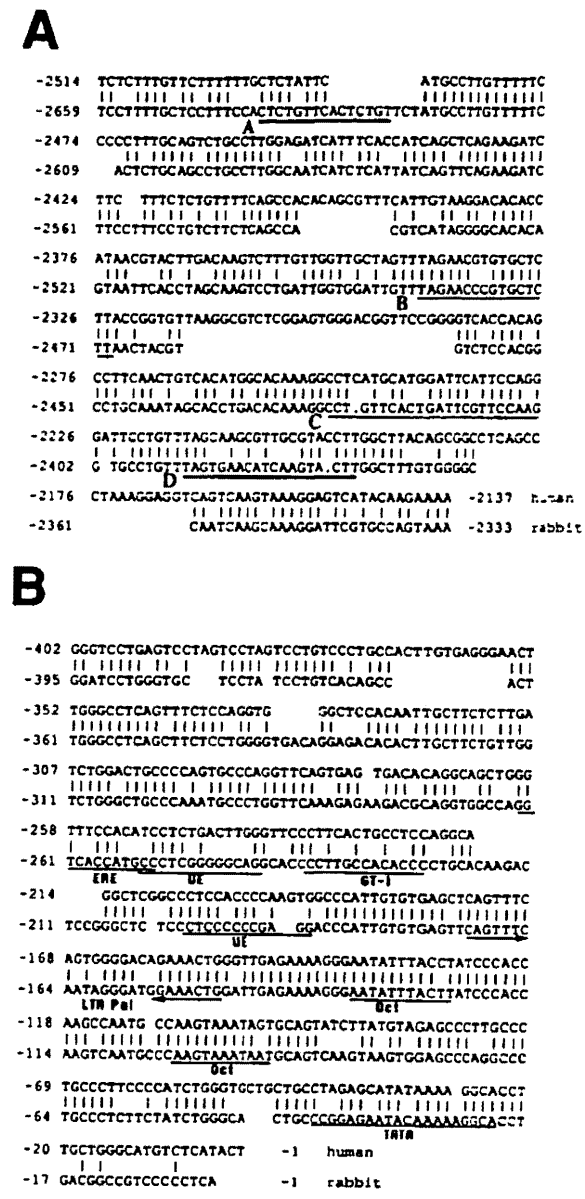

Figure 4. Sequence alignments of homologous human CC10 and rabbit uteroglobin 5'-flanking gene sequences using the GCG program 'Best Fit'. A, The human CC10 gene sequence (upper sequence) from −2,514 to −2,137 bp is aligned to the rabbit uteroglobin 5'-flanking gene sequence (lower sequence) from −2,659 to −2,333 bp. In the rabbit gene, the two strong (A and D) and the two weak (B and C) progesterone receptor binding sites according to Jantzen et al. (32) are underlined. B, The promoter region of the rabbit uteroglobin gene (lower sequence) from −395 to −1 bp is aligned to the corresponding human CC10 gene region (upper sequence). Recently identified *cis*-elements of the rabbit promoter (33,44) are underlined and designated ERE (estrogen responsive element), GT-I (GT-I motif found in the SV40 enhancer), LTR Pal (palindromic sequence found in the long terminal repeat of murine leukemia viruses), Oct (sequence motifs that bind purified Oct-1 specifically) and the TATA region (TATA box).

promoters (45) and do bind purified Oct1 specifically (unpublished results) are conserved in the human promoter around −100 bp and −135 bp. Most interestingly, in DNaseI footprinting experiments a protection over the rabbit uteroglobin TATA-box

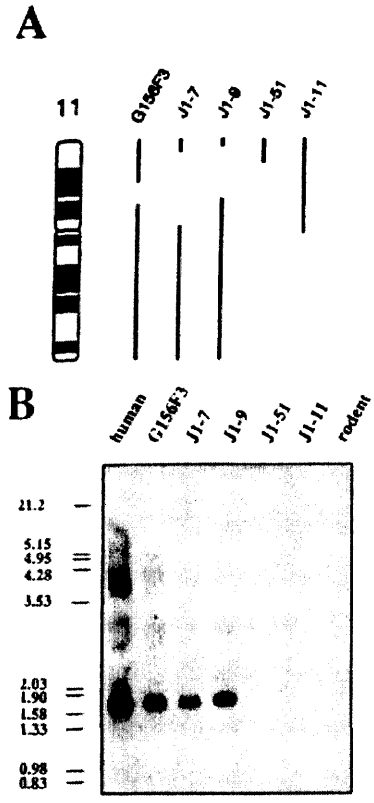

Figure 5. Subregional chromosomal mapping of the human CC10 gene. A, Schematic drawing of human chromosome 11 (left) and remaining chromosome 11 fragments (indicated by vertical lines) in various somatic cell hybrid lines (G156F3, J1-7, J1-9, J1-51 and J1-11). B, Southern blot containing 10 μg of Eco RI/Hind III restricted genomic DNA from various cell lines was hybridized with a 1.7 kb Eco RI/Hind III fragment (see Fig. 2A) under standard conditions. Lanes: 1, human control DNA; 2, G156F3 hybrid cell line (del prox11p14-dist11p12) (59); 3 to 6, J1 derived somatic cell hybrid lines J1-7 (lane 3), J1-9 (lane 4), J1-51 (lane 5), J1-11 (lane 6) as described in Glaser et al. (47); 7, rodent control DNA.

has been observed exclusively with extracts from Ishikawa cells indicating that a tissue-specific factor binds to the TATA-box region (44). This region is in 15 out of 20 nucleotides conserved in the human gene.

Chromosomal localization of the human CC10 gene

To determine the chromosomal localization of the human CC10 gene a genomic 1.7 kb Eco RI-Hind III fragment spanning the transcriptional start point (see Fig. 2A) was hybridized to a human-mouse somatic cell hybrid panel (46). Scoring for the presence of the specific hybridization signal led to an initial assignment to chromosome 11. This result was confirmed using a subregional mapping panel based on cell lines derived from a human chromosome 11 unique hybrid (47). The human CC10 gene is present in the cell lines J1-7 and J1-9 that carry complete chromosome 11q but have different 11p deletions. In contrast, it is absent in the cell line J1-11, which retained 11pter-11q11 (Fig. 5). From these data we conclude that the human CC10 gene is localized in the chromosome11q11-qter region.

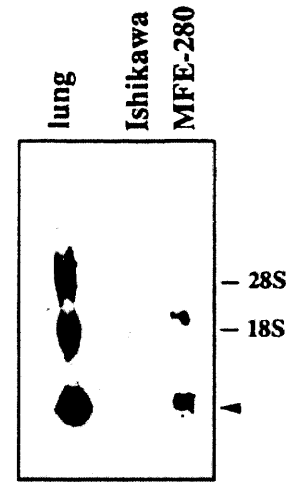

Figure 6. Human CC10 mRNA expression. Northern blot analysis: Total RNA from lung (4 μg) and poly (A^+) RNA from Ishikawa (4 μg) and MFE-280 cells (4 μg) were electrophoresed through 1.5% agarose, blotted to a nylon membrane and hybridized to ^{32}P-labelled human uteroglobin cDNA. The positions of the 28S and 18S markers are indicated. The arrow depicts the uteroglobin mRNA signal.

Human CC10 is expressed in a cell line of endometrial origin

Human CC10 was originally isolated from lung Clara cells and therefore designated 10 kDa Clara cell secretory protein. In addition to this bronchiolar cell type, rabbit uteroglobin is expressed in certain epithelial cells of the endometrium, oviduct, male genital tract and esophagus. When we analyzed total RNA from five different samples of human endometrial tissue, we could not detect any CC10 mRNA (data not shown). In contrast, human CC10 mRNA is detectable in the cell line MFE-280 (Fig. 6) that has been recently established from a human adenocarcinoma of endometrial origin (to be published elsewhere). Furthermore, by Northern blot analysis human CC10 mRNA is not detectable in another cell line, the Ishikawa cell line (48). The amount of CC10 mRNA in MFE-280 cells is very low compared to the lung. The signal we obtain with 4 μg total lung RNA is approximately 4-fold stronger compared to the signal obtained with 4 μg of poly$(A)^+$ RNA from the MFE-280 cell line. As only 4% of all lung cells (the Clara cells lining the bronchiolar epithelium) express CC10 (49) we estimate that the amount of human CC10 mRNA per cell in the MFE-280 cell line is approximately 1,000-fold lower than in lung Clara cells *in vivo*. Rabbit uteroglobin mRNA expression in rabbit endometrium is strongly inducible by sequential treatment with estrogen and progesterone. As the MFE-280 cell line does not contain significant amounts of the corresponding steroid hormone receptors (unpublished results) we could not analyze the hormonal inducibility of the human CC10 mRNA in this cell line.

DISCUSSION

A human cDNA clone coding for a 91 amino acid protein that shows 61% identity to rabbit uteroglobin on the protein level has been previously isolated and designated as 10 kDa Clara cell secretory protein (CC10) according to its expression in lung Clara

cells (8) or as 17 kDa Clara cell secretory protein which indicates the correct molecular weight (1). Further structural and functional features of the protein suggest that it is the human homologue of rabbit uteroglobin. Nevertheless, it was argued that CC10 may not be uteroglobin (5). To clarify this point, the amount of divergence at synonymous (silent) and nonsynonymous sites between human CC10, rat CC10 and rabbit uteroglobin coding regions was analyzed. Our data show that human CC10 and rabbit uteroglobin are more closely related to each other than to rat CC10, thus providing good evidence for a one gene hypothesis. The finding that the average numbers of substitutions per site for the uteroglobin/CC10 gene are higher than for many other genes shows that it is evolving at higher speed than average. Most likely this is a consequence of only moderate constraints by the function(s) of the protein on its primary structure. Moreover, Southern blot analysis indicates that there exists only a single CC10 gene in man ((8) and our own unpublished results). Therefore, to circumvent different misleading nomenclatures we propose to designate the human as well as the rat homologues (6,7) of rabbit uteroglobin as uteroglobins according to the designation in the species where it was first described.

Human uteroglobin/CC10 mRNA is strongly expressed in lung Clara cells. In addition, by Northern blotting the mRNA is detectable in a cell line (MFE-280) that has been established from a human endometrium carcinoma. This is the first report of endometrial cells that express endogenous uteroglobin/CC10 mRNA indicating that human uteroglobin/CC10 is expressed in endometrium as is its rabbit homologue. Thus, it appears that human uteroglobin/CC10 is not only a marker for lung Clara cells but also an endometrial differentiation marker. Human uteroglobin/CC10 cDNA or antibodies to the protein may be used to characterize endometrium derived tumors. Several reasons might account for the finding that human uteroglobin/CC10 mRNA is expressed in an endometrium-derived cell line but has not been detectable in a series of normal tissue samples from uterus. As rabbit and murine uteroglobin/CC10 are expressed exclusively in the luminal and the glandular epithelium (5,28) it is likely that human uteroglobin/CC10 mRNA expression is also restricted to these epithelial layers. We have extracted and analyzed total RNA from whole organs and, therefore, it is possible that we have been below the detection level. Alternatively, human uteroglobin/CC10 mRNA might not be expressed constitutively in the uterus during the menstrual cycle but regulated by steroid hormones similarly to rabbit (23) or rat (7) and mouse (35) uteroglobin/CC10. Thus, it is conceivable that uteroglobin/CC10 expression in the uterus is restricted to a very short time period of the cycle. Some evidence for such a cycle-dependent expression comes from a previous report. A human protein cross-reactive to a monospecific anti-rabbit uteroglobin antiserum has been detected in some uterine washings obtained from women during the early and midluteal phases of the ovarian-menstrual cycle, but not in uterine washings from other menstrual phases (50).

Experiments addressing the regulation of human uteroglobin/CC10 mRNA expression in endometrium and lung by steroid hormones could not be performed as the endometrium derived cell line MFE-280 does not contain measurable amounts of steroid hormone receptors and appropriate human uteroglobin-expressing lung cell lines are not available yet. Thus, it remains unclear whether human uteroglobin/CC10 mRNA expression can be modulated by steroid hormones as described for the rabbit tissues. In this species, uteroglobin mRNA expression in endometrium can be induced by sequential treatment of estrogen and progesterone (23) and in lung glucocorticoids increase the amount of uteroglobin mRNA specifically. Sequence alignments of the human with the rabbit uteroglobin 5'-flanking regions do not point to a similar regulation by steroid hormones. The well defined estrogen responsive element found in the rabbit uteroglobin promoter at position −263/−252 (33) is not conserved in the human gene despite the overall homology in the promoter region. The cluster of progesterone receptor binding sites found in the rabbit uteroglobin 5'-flanking gene region is only partially conserved in the human gene. Close inspection of the sequence reveals that only two weak binding sites are partially conserved (32). It remains to be established whether these two partially conserved elements can still bind the progesterone receptor and can function as progesterone responsive elements. In the rat 5'-flanking uteroglobin gene region the hormone receptor binding sites described for the rabbit gene are also absent. Nevertheless, expression of uteroglobin/CC10 mRNA in rat uterus is inducible with estrogen/progesterone (7) and in lung cortisol modulates uteroglobin mRNA expression (7). Therefore, the absence of the discussed steroid hormone receptor binding sites in the human 5'-flanking gene region does not necessarily indicate that expression of the gene is not regulated by steroid hormones. Still unidentified hormone responsive elements or an indirect effect of the hormones may account for these observations. A computer search for potential glucocorticoid/progesterone receptor binding sites revealed five half palindromic recognition sites (TGTYCT) located at positions −2509/−2504, −2382/−2387, −1018/−1023, −804/−809 and −777/−782.

Comparison of the human uteroglobin/CC10 promoter to the rabbit uteroglobin promoter reveals that elements from −258 to −220 bp and from −205 to −177 bp that are very important for the transient activity of the rabbit promoter in Ishikawa cells (44) are not conserved in the human promoter. In contrast, the sequence further downstream from −180 bp to the TATA box is well conserved between human and rabbit. In that region at least 4 distinct footprints have been observed in DNaseI protection experiments (44). The significance of these and other *cis*-elements for the cell type-specific expression of the uteroglobin genes remains to be elucidated in gene transfer experiments. *In vitro* transcription experiments with Ishikawa cell nuclear extracts have already shown that the region between −96 and −35 bp is important for the activity of the rabbit promoter. To further narrow down the relevance of the promoter sequence that is conserved between the rabbit and human uteroglobin/CC10 genes we have generated linker scanning mutations in the appropriate promoter region and are currently investigating these mutants in gene transfer experiments. For these kind of experiments the MFE-280 cell line will be very useful.

MATERIALS AND METHODS

Cell culture

Ishikawa cells were cultured as described previously (33). MFE-280 cells were derived from a recurrent endometrium carcinoma. The tumor was placed at the pelvic wall and classified as a poorly differentiated adenocarcinoma. The cells were grown in suspension in DMEM medium, supplemented with 10% FCS, 40 IU/l insulin, 2.5 mg/l transferrin, 10 nM 17β-estradiol and 67 μg/ml gentamycine sulfate.

Northern blot analysis

Total RNA from human lung and cell lines was extracted by the guanidinium/phenol procedure (51). Poly (A^+) RNA was isolated by oligo (dT)-cellulose chromatography using the QuickPrep mRNA Purification Kit from Pharmacia. Total and poly (A^+) RNA were separated on 1.5% agarose gels containing 2.2 M deionized formaldehyde and blotted to nylon membranes (Pall Biodyne B). Prehybridization and hybridization was carried out as described (7) using ^{32}P labelled human CC10 cDNA (8) as probe. The specific activity of the probe was approximately 5×10^8 cpm/μg.

S1 nuclease mapping

S1 nuclease mapping was carried out as described (52,53). A 639 bp EcoRI-PstI fragment from plasmid clone phUGprom (see 'Gene isolation and mapping' and Fig. 2A) was dephosphorylated and end-labelled with T4 polynucleotide kinase and [γ-^{32}P]ATP according to standard procedures (54). After digestion with SacI, the resulting 283 bp PstI-SacI fragment spanning the presumed transcriptional start site was isolated and hybridized to total RNA from human lung and Ishikawa cells, respectively. Hybridization was performed with 10,000 cpm and 10 μg total RNA in 40 mM Pipes, pH 6.5, 0.4 M NaCl, 1 mM EDTA, 80% formamide at 42°C overnight. After 5-fold dilution with 30 mM sodium acetate, 3 mM zinc acetate, S1 nuclease digestions were carried out at 30°C for 45 min with various S1 nuclease concentrations as indicated in the legend to Fig. 2B. The reaction was stopped with 15 mM EDTA, phenol extracted and ethanol precipitated prior to electrophoresis

Southern blot analysis

High-molecular-mass DNA was isolated and purified from hybrid cell lines according to standard procedures (54). The DNA (10 μg) was digested with Eco RI/Hind III and separated on 0.8% agarose gels. After blotting to nylon membranes prehybridization and hybridization were carried out as described for the Northern blot analysis.

Gene isolation and mapping

A commercially available human genomic λ fix phage library (Stratagene) prepared from the human lung fibroblast cell line W1 38 was screened with human CC10 cDNA (8). One positive clone was identified. After three rounds of rescreening, bacteriophage DNA was prepared from single plaques according to Grossberger (55). A partial restriction map was obtained by single and double digestions with Bam HI, Eco RI and Hind III, separation through 0.8% agarose and Southern blot analysis (54) using subfragments of the human CC10 cDNA as probes. To facilitate further mapping and sequence analysis of the promoter region a 4 4 kb Bam HI-Hind III fragment that hybridized exclusively to a 60 bp Eco RI-Pst I human CC10 cDNA subfragment containing the codons for the 16 most NH_2-terminal amino acids (8) was subcloned into the plasmid vector pSPT19 (phUGprom).

DNA sequencing

The dideoxy chain termination method (54) was employed for sequence determination using the Pharmacia T7 sequencing kit and [α-^{35}S] dATP (specific activity: 22 TBq/mmol) throughout. Plasmids were denatured with alkali and hybridized to chemically synthesized 17 to 26-mer oligonucleotide primers for plasmid and insert-specific sequences. For low level analysis of sequences the computer program DNA Strider 1.1 (56) was used.

ACKNOWLEDGEMENTS

Dr M.Kalff-Suske is gratefully acknowledged for critical reading of the manuscript. We thank Dr. P.Sharp for discussions and providing the computer program LWL90 written by Dr. K.H.Wolfe. Drs. S.L.Katyal and G.Singh kindly provided the human CC10/uteroglobin cDNA. This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Mu601/5-1) and by the Fonds der chemischen Industrie.

REFERENCES

1. Umland,T.C., Swaminathan,S., Furey,W., Singh,G., Pletcher,J. and Sax,M. (1992) *J. Mol. Biol.* **224**, 441–448.
2. Singh,G. and Katyal,S.L. (1984) *J. Histochem. Cytochem.* **32**, 49–54.
3. Singh,G., Singal,S., Katyal,S.L., Brown,W.E. and Gottron,S.A. (1987) *Exp. Lung Res.* **13**, 299–309.
4. Nordlund-Möller,L., Andersson,O., Ahlgren,R., Schilling,J., Gillner,M., Gustafsson,J.-A. and Lund,J. (1990) *J. Biol. Chem.* **265**, 12690–12693.
5. Singh,G., Katyal,S.L., Brown,W.E., Kennedy,A.L., Singh,U. and Wong-Chong,M.-L. (1990) *Biochim. Biophys. Acta*, **1039**, 348–355.
6. Katyal,S.L., Singh,G., Brown,W.E., Kennedy,A.L., Squeglia,N. and Wong Chong,M.-L. (1990) *Prog. Respir. Res.*. **25**, 29–35.
7. Hagen,G., Wolf,M., Katyal,S.L., Singh,G., Beato,M. and Suske,G.(1990) *Nucleic Acids Res.* **18**, 2939–2946.
8. Singh,G., Katyal,S.L., Brown,W.E., Phillips,S , Kennedy,A.L., Anthony,J. and Squeglia, N.(1988) *Biochim. Biophys. Acta* **950**, 329–337.
9. Morize,I., Surcouf,E., Vaney,M.C., Epelboin,Y., Buehner,M., Fridlansky,F., Milgrom,E. and Mornon,J.P. (1987) *J. Mol. Biol.* **194**, 725–739.
10. Beier,H.M. (1968) *Biochem. Biophys. Acta.* **160**, 289–291.
11. Kirchner,C. (1976) *Cell Tissue Res.* **170**, 490–492.
12. Beier,H.M., Bohn,M and Müller,W. (1975) *Cell Tissue Res.* **165**, 1–11.
13. Noske,I.G. and Feigelson,M. (1976) *Biol. Reprod.*, **15**, 704–713.
14. Torkkeli,T., Krusius,T. and Jänne,O. (1978) *Biochem. Biophys. Acta*, **544**, 578–592.
15. Beato,M. and Baier,R. (1975) *Biochim. Biophys. Acta* **392**, 346–356.
16. Beato,M., Arnemann,J. and Voss,H.J. (1977) *J. Steroid Biochem.* **8**, 725–730.
17. Atger,M., Savouret,J.F. and Milgrom,E. (1980) *J. Steroid Biochem.* **13**, 1157–1162.
18. Gillner,M., Lund,J., Cambillau,C., Alexandersson,M., Hurtig,U., Bergman,A., Klasson-Wehler,E. and Gustafsson,J.A. (1988) *J. Steroid Biochem.* **31**, 27–33.
19. Lund,J., Brandt,I., Poellinger,L., Bergman,A., Klasson-Wehler,E. and Gustafsson,J.A. (1985) *Mol. Pharmacol.* **27**, 314–323.
20. Levin,S.W., Butler,J.D., Schumacher,U.K., Wightman,P.D. and Mukherjee,A.B. (1986) *Life Sci.* **38**, 1813–1819.
21. Miele,L., Cordella-Miele,E. and Mukherjee,A.B. (1987) *Endocr. Rev.* **8**, 474–490.
22. Miele,L , Cordella-Miele,E., Facchiano,A. and Mukherjee,A.B. (1988) *Nature* **335**, 726–730.
23. Beato,M., Arnemann,J., Menne,C., Müller,H., Suske,G. and Wenz,M. (1983) In McKerns,K.W. (ed.), Regulation of Gene Expression by Hormones. Plenum Press, New York, pp. 151–175.
24. Müller,H. and Beato,M. (1980) *Eur. J. Biochem.*, **112**, 235–241.
25. Lopez de Haro,M.S., Alvarez, L. and Nieto,A. (1988) *Biochem. J.* **250**, 647–651.
26. Fernandez-Renau,D., Lombardero,M. and Nieto,A. (1984) *Eur. J. Biochem.* **144**, 523–527.
27. Lopez de Haro,M.S. and Nieto,A. (1985) *Biochem. J.* **225**, 255–258.
28. Warembourg,M., Tranchant,O., Atger,M. and Milgrom,E. (1986) *Endocrinology* **119**, 1632.
29. Menne,C., Suske,G., Arnemann,J., Wenz,M., Cato,A.C.B. and Beato,M. (1982) *Proc. Natl. Acad. Sci.* USA, **79**, 4853–4857.
30. Suske,G., Wenz,M., Cato,A.C.B. and Beato,M. (1983) *Nucleic Acids Res.* **11**, 2257–2271.
31. Cato,A.C.B., Geisse,S., Wenz,M., Westphal,H.M. and Beato,M. (1984) *EMBO J.* **3**, 2771–2778.
32. Jantzen,K., Fritton,H.P., Igo-Kemenes,T., Espel,E., Janich,S., Cato,A.C.B., Mugele,K and Beato,M. (1987) *Nucleic Acids Res.* **15**, 4535–4552.
33. Slater,E.P., Redeuihl,G., Theis,K., Suske,G. and Beato,M. (1990) *Mol. Endocrinol.* **4**, 604–610.
34. Nieto,A. and Lombardero,M. (1982) *Comp. Biochem. Physiol. B* **71**, 511–514.
35. Sandmöller,A., Voss,A.K., Hahn,J., Redemann-Fibi,B., Suske,G. and Beato,M. (1991) *Mechanisms of Development* **34**, 57–68.
36. Lopez de Haro,S. and Nieto, A. (1986) *Biochem. J.* **235**, 895–898.
37. Li,W.-H., Tanimura,M., Luo,C.-C., Datta,S. and Chan,L. (1988) *J. Lipid Res.* **29**, 245–271.
38 Li,W.-H., Gouy,M., Sharp,P.M., O'hUigin,C. and Yang,Y.-W. (1990) *Proc. Natl. Acad. Sci.* USA **87**, 6703–6707.
39. Bulmer,M., Wolfe,K.H. and Sharp,P.M. (1991) *Proc. Natl. Acad. Sci.* USA **88**, 5974–5978.
40. Sollner-Webb,B. and Reeder, R.H. (1979) *Cell* **18**, 485–499.
41. Devereux,J., Haeberli,P. and Smithies,O. (1984) *Nucleic Acids Res.* **12**, 387–395.
42. Schmid,C.W. and Jelinek,W.R. (1982) *Science*, **216**, 1065–1070.
43. Ullu,E. and Tschudi,C. (1984) Nature, **312**, 171–172.
44. Misseyanni,A., Klug,J., Suske,G. and Beato,M. (1991) *Nucleic Acids Res.* **19**, 2849–2859.

45. Rosenfeld,M.G. (1991) *Genes & Dev.* **5**, 897–907.
46. Willecke,K., Jungbluth,S., Dahl,E., Hennemann,H., Heynkes,R. and Grzeschik,K.H. (1990) *Eur. J. Cell Biol.* **53**, 275–280.
47. Glaser,T., Housman,D.E., Lewis,W.H., Gerhard,D. and Jones,C. (1989) *Somatic Cell Mol. Genetics* **15**, 477–501.
48. Nishida,M., Kasahara,K., Kaneko,M. and Iwasaki,H. (1985) *Acta Obstet.Gynaec. Jap.* **37**, 1103–1111.
49. Singh,G., Singh,J., Katyal,S.L., Brown,W.E., Kramps,J.A., Paradis,J.L., Dauber,J.H., Macpherson,T.A. and Squeglia,N. (1988) *J. Histochem. Cytochem.* **36**, 73–80.
50. Cowan,B.D., North,D.H., Whitworth,N.S., Fujita,R., Schumacher,U.K. and Mukherjee,A.B. (1986) *Fertil. Steril.* **45**, 820–823.
51. Chomczynski,P. and Sacchi,N. (1987) *Anal. Biochem.* **162**, 156–159.
52. Berk,A.J., and Sharp,P.A. (1977) *Cell* **12**, 721–732.
53. Weaver,R.F. and Weissmann,C. (1979) *Nucleic Acids Res.* **7**, 1175–1193.
54. Maniatis,T., Fritsch,E.F., Sambrook,J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor.
55. Grossberger,D. (1987) *Nucleic Acids Res.* **15**, 6737.
56. Marck,C. (1988) *Nucleic Acids Res.* **16**, 1829–1836.
57. Li,W.-H., Wu,C.-I. and Luo,C.-C. (1985) *Mol. Biol. Evol.* **2**, 150–174.
58. Higgins,D.G. and Sharp,P.M. (1988) *Gene* **73**, 237–244.
59. Gessler,M., Thomas,G.H., Coullin,P., Junien,C., McGillivray,B.C., Hayden,M., Jaschek,G. and Bruns,G.A.P. (1989) *Amer. J. Hum. Genet.* **44**, 486–495.