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Molecular analysis of the ovine cystic fibrosis transmembrane conductance regulator gene

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ABSTRACT **There is a need for a large-animal model to investigate the etiology and biology of cystic fibrosis (CF) lung disease and to study potential therapies. The development and electrophysiology of the sheep airway have been shown to exhibit close functional parallels with the human airway, particularly with respect to the respiratory epithelium. We have cloned and sequenced the ovine cystic fibrosis transmembrane conductance regulator (CFTR) cDNA. It shows a high degree of conservation at the DNA coding and predicted polypeptide levels with human CFTR: at the nucleic acid level there is a 90% conservation (compared with 80% between human and mouse CFTR cDNA); at the polypeptide level, the degree of similarity is 95% (compared with 88% between human and mouse). Northern blot analysis and reverse transcription–PCR have shown that the patterns of expression of the ovine CFTR gene are very similar to those seen in humans. Further, the developmental expression of CFTR in the sheep is equivalent to that observed in humans. Thus, overall a CF sheep should show lung pathology similar to that of humans with CF.**

The autosomal recessive disease cystic fibrosis (CF), which is characterized by severe lung disease, digestive problems, male infertility, and an elevated sweat chloride ion concentration, is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene (1, 2). The CFTR protein codes for a small conductance chloride ion channel (3–7), although judging from its homology with members of the ABC transporter superfamily (8), it may have one or more, as yet unknown, additional functions. Despite this incomplete understanding of the function of the CFTR gene and its protein product, substantial progress has been made toward devising strategies for CF gene therapy. In the absence of a naturally occurring animal model for CF, four lines of transgenic CF mice have been generated. Each of these carries a different mutation: one contains an insertion mutation (9), two have disruptions within exon 10 of the mouse gene (10–12), and one has a targeted disruption of exon 3 (13). All transgenic CF mice show some pathological features that are reminiscent of the pathology of CF in humans; however, the respiratory system and pancreatic ducts are minimally affected. In general, the mice show severe intestinal disease, and it is this aspect of the pathology that proves lethal, rather than the lung disease that is usually responsible for death in humans with CF. This is possibly due to anatomical and physiological differences in the human and mouse lung, including the presence in some epithelia of alternative routes for chloride secretion (14, 15). Lung epithelium in transgenic CF mice does show electrophysiological differences from normal mouse lung epithelium, and it appears possible to correct these electrophysiological differences by gene therapy (16, 17). Treatment of CF lung disease may also benefit from pharmacological approaches to treatment; however, due to species differences, particularly in body size and lung anatomy, it may not be possible to test these treatments effectively on mice.

Another aspect of the CF disease process that may not be adequately addressed by the CF mouse model is the developmental expression of the disease. It has been shown that the CFTR gene is expressed early in human development (18, 19), and this correlates temporally with the development of CF pathology, particularly in the pancreas. Importantly, CFTR mRNA, which is expressed at low levels in adult respiratory epithelium, is present at substantial levels throughout the respiratory epithelium prior to birth.

A large animal model of CF would be a great advantage in investigating the significance of the developmental expression of CFTR and furthering pharmacological approaches to the treatment of CF lung disease. Such an animal would be sufficiently large to allow bronchoscopy and survive lung biopsy and suitably long-lived to allow treatment to be followed for up to a decade. The sheep is an ideal potential model for CF study, as its lung has been well studied from both electrophysiological and developmental aspects and shows close similarities to the human lung, particularly with respect to the function of the respiratory epithelium (20, 21). We have embarked on a project to breed a sheep with CF. As a first step, we have cloned the ovine CFTR gene and shown that its pattern of expression is similar to that of the human CFTR gene.§

MATERIALS AND METHODS

Tissue Samples. Sheep tissue samples were collected from the following developmental stages: midfetal tissues were of 70 days gestation, equivalent to an 18-week human fetus (the gestation period of a sheep being 148 days); late-fetal tissues were of 138 days gestation, about 10 days before birth; and adult tissues, including blood, were obtained from pregnant ewes from 4 to 6 years of age. Tissues were immediately frozen and stored in liquid nitrogen. Prior to lymphocyte preparation (see below), 50 mg of EDTA was added to 10-ml aliquots of whole blood.

Extraction of RNA from Sheep Tissues and Lymphocytes. Lymphocytes were isolated from whole blood by Histopaque (Sigma) centrifugation. Cells were washed in phosphate-buffered saline, and then poly(A)$^+$ RNA was extracted by using a QuickPrep Micro mRNA Purification Kit (Pharmacia). Total RNA was isolated from sheep tissue samples by lysis in guanidinium isothiocyanate followed by CsCl centrifugation (22). Poly(A)$^+$ RNA was isolated from total RNA by using

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Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; CF, cystic fibrosis; RT-PCR, reverse transcription–PCR; MSH, membrane-spanning helix; R domain, regulatory domain; NBD, nucleotide-binding domain.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. U20418).

oligo(dT) Dynabeads (Dynal, Oslo), according to the method of Jakobsen *et al.* (23).

Reverse Transcription–PCR (RT-PCR). Specific fragments of the ovine CFTR cDNA were amplified either from lymphocyte poly(A)$^+$ RNA or from midfetal tissue total RNA by the method of Chalkley and Harris (24), which has been shown to detect the functional CFTR sequence in humans. The primer sequences are shown in Table 1. For the PCR reaction, an initial 5-min denaturation step at 94°C was followed by 30 cycles of 1 min at 94°C (denaturation), 1 min at 60°C (annealing), and 4–7 min at 72°C (extension using AmpliTaq; Perkin–Elmer), the time depending on the length of the cDNA fragment. After the 30 cycles, a final polymerization step was carried out at 72°C for 5 min. For amplification from lymphocyte RNA, two rounds of PCR were required. A total of 1 μl of the first PCR reaction mixture was transferred to 49 μl of a PCR buffer mixture containing 500 ng of each primer in the nested set. An additional 30 cycles of amplification were performed by using the same parameters as for the first reaction.

For RT-PCR analysis of CFTR gene expression, a 530-bp fragment of the expressed P1 housekeeping gene encoding subunit c of sheep mitochondrial ATP synthase (25) was coamplified with fragment C (792 bp) of the ovine CFTR mRNA by using RT-PCR on total RNA. The primers homologous to the P1 housekeeping gene were at 1/10th the concentration of those for CFTR. The specific fragments generated by each PCR were verified by restriction enzyme digestion and direct sequencing. All primer sets were shown not to amplify a specific product from genomic DNA.

Cloning and Sequencing of the Ovine CFTR cDNA Fragments. PCR products were isolated by using a Geneclean II kit (Stratech, London). Purified PCR product (100–200 ng) was subjected to direct sequence analysis (26) by using deoxyadenosine 5′-[γ-[^{35}S]thio]-triphosphate, Sequenase version 2.0 DNA polymerase (United States Biochemical), and an appropriate oligonucleotide primer to verify the product as CFTR. Another aliquot of the PCR product was then inserted into the pCRII vector by using the TA Cloning System (Invitrogen). DNA sequencing was carried out by using the dideoxynucleotide chain-termination method of Sanger *et al.* (27) as adapted for double-stranded templates by Murphy and Kavanagh (28). Templates were primed by using the M13 forward and reverse primer oligonucleotides. DNA sequence information was assembled and analyzed by using computer software (29, 30). Three independent RT-PCR clones were characterized for each ovine CFTR cDNA fragment. All DNA sequence information was obtained from both strands.

Northern Analysis. Total RNA (20 μg) isolated from fetal and pregnant adult sheep tissues was heated at 60°C for 15 min in a solution of 50% deionized formamide/2% formaldehyde/0.02 M Mops. Glycerol/dye buffer was added, and the RNA was fractionated at 45 V overnight on a 1.5% agarose gel containing 0.02 M Mops and 6% formaldehyde. RNA size markers (GIBCO/BRL) were treated in an identical manner. The RNA was transferred onto Hybond-N membrane (Amersham) by standard methods and probed with randomly primed ^{32}P-labeled ovine CFTR fragment D′ (Megaprime DNA labeling kit; Amersham).

RESULTS AND DISCUSSION

Cloning and Sequencing the Ovine CFTR cDNA. Nested RT-PCR was used to amplify specific regions A–F of the ovine CFTR cDNA, which broadly correspond to functional domains of the CFTR protein, from sheep blood lymphocyte poly(A)$^+$ RNA by the methodology of Chalkley and Harris (24). Amplified DNA fragments were gel purified and directly sequenced to confirm their CFTR identity before being inserted into the pCRII vector. The specific regions A, B, C, D′, and F were amplified and cloned on the basis of sequence similarity with human CFTR primer sequences or, in the case

Table 1. Details of primers used in the amplification and cloning of ovine CFTR cDNA fragments

Clone identifier Size, bp Boundaries, nt	First and second round PCR primers and positions
α	AST4R* 1 to 19
	TGGAAGCAAATGACATCAC
678	AST1L† 678 to 661
1 to 678	GAGACTAACAAGTTGTCC
A	A1R 121 to 140
	CgAGAGACCATGCAGAGGTC
1010;	A1L† 1229 to 1210
153 to 1162	GCTCCAAGAGAgTCATACCA
	A2R‡ 153 to 172
	GGCCAGCGTtGTCTCCAAAC
	A2LA‡ 1162 to 1145
	CAATGCAGAATGAGATGG
B	B1R 995 to 1014
	GACAAACAGAACTGAAACTg
896;	B1L† 1966 to 1947
1019 to 1914	CAGCTTTCTTTAAaTGTTCC
	B2R‡ 1019 to 1038
	GGAAGGCAGCCTATGTGAGA
	B2L‡ 1914 to 1895
	AGCCATCAgTTTACAGACACCAGC
C	C1R 1772 to 1791
	GTGGAGGTCAaCGAGCAAGA
792;	C1L† 2623 to 2604
1806 to 2597	AcTCCtTTAAgTCTTCTTCg
	C2R‡ 1806 to 1825
	AGCAGTATACAAAGATGCTG
	C2L‡ 2597 to 2578
	TCTTCACTTATTTCCAAGCC
D′	D1RB 2427 to 2448
	GTCAGTTCTGAACCTTATGACC
1455;	E1LB† 3991 to 3969
2472 to 3926	CAAAGGCCTTCCTCCATTGTTGC
	D2RB‡ 2472 to 2493
	GAGCATTCATCGAAAGACAGCG
	E2LB‡ 3926 to 3906
	CCTTTGGTATTCAGCAGTCTC
F	F1R 3722 to 3741
	AcGTgAAGAAAGATGACATC
785;	F3L† 4573 to 4554
3762 to 4546	AaAGCcTTGTaTCtTGCACC
	F2R‡ 3762 to 3781
	GACTGTCAAAGAtCTCACAG
	F4L‡ 4546 to 4526
	CTGTCTCCTCtTTCAGAGCaG
G	FST3R 4338 to 4357
	CAGGATAGAAGCAATGTTGG
458;	GST1L*† 4795 to 4776
4338 to 4795	CAAGTGGTAAATCTTCAAGG

Primer sequences were based on human CFTR for fragments A, B, C, and F; on ovine sequence from C and F for D′; on human/mouse homology and ovine sequence for α; on ovine and human/bovine homology for G. cDNA fragments A–F were cloned from adult sheep lymphocyte poly(A)$^+$ RNA, α and G from midterm ovine fetal pancreas and colon total RNA. Sequence in boldface type is ovine sequence. Sequence not in boldface type is from nonovine source. Lowercase sequence in primers represents a mismatch with ovine CFTR.
*AST4R and GST1L; primer sequence derived from human and bovine CFTR sequence homology, not confirmed as ovine sequence.
†First round primer used for reverse transcription.
‡Second round primer.

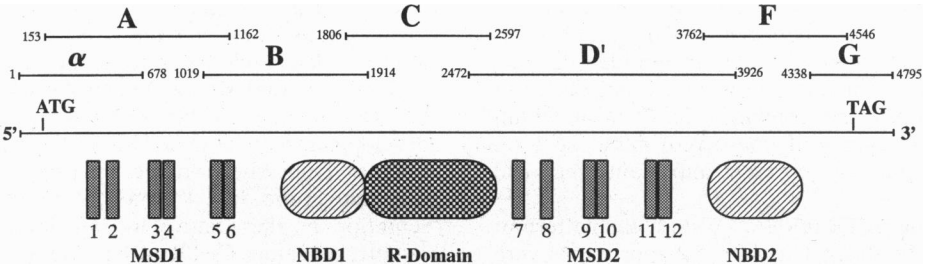

FIG. 1. Schematic diagram showing the relationships among the ovine CFTR-specific clones and the entire CFTR cDNA. α, A, B, C, D′, F, and G represent each of the cloned fragments, with the numbering referring to their positions with respect to the nucleotide sequence of full-length CFTR cDNA. D′ is equivalent to a combination of the human CFTR cDNA fragments D and E (24). The translational start codon (ATG) and stop codon (TAG) are shown. Below the cDNA is a representation of the functional domains of the CFTR protein: MSD, membrane-spanning domain; NBD, nucleotide-binding domain; R-Domain, regulatory domain. Each of the MSDs is divided into six membrane-spanning helices (MSHs).

of D′ (corresponding to a combination of the human CFTR cDNA fragments D and E; ref. 24), with ovine CFTR sequences previously characterized in regions C and F (see Table 1).

The 5′-(α) and 3′-(G) specific regions of the ovine CFTR cDNA were amplified by RT-PCR from RNA extracted from ovine fetal pancreas and colon tissues. Nested PCR was not required since the tissues selected, in contrast to lymphocytes, express the CFTR gene at relatively high levels. The primer sequences for the α and G regions were derived from ovine CFTR sequence information from A and F and also on CFTR sequence homology among human (GenBank: HUMCFTRM, M28668; ref. 2), mouse (GenBank: MUSCFTR, M69298; ref. 31), and cow (GenBank: BOVTCRCF, M76128; ref. 32). Amplified α and G fragments were identified as ovine CFTR and inserted into the pCRII vector.

Three independent RT-PCR amplified clones from each of the α, A, B, C, D′, F, and G regions were sequenced and characterized. Sequence discrepancies among the three clones were interpreted mainly as *Taq* polymerase-induced errors, given that *Taq* polymerase lacks proofreading ability and is prone to introduce nucleotide base errors that are amplified as PCR proceeds (33). All sequence discrepancies were eliminated by direct sequence analysis of genomic DNA.

The consensus sequence from each of the seven fragments was ordered and assembled into the overall ovine CFTR cDNA sequence [GenBank accession no. U20418 (OVCFTRC)]. The spatial relationships among the seven fragments, as well as the location of the functional domains of the CFTR polypeptide, are represented schematically in Fig. 1. The predicted 1481-amino acid sequence of the ovine CFTR protein is shown in Fig. 2.

The compiled sequence contains the entire ovine CFTR coding region (nucleotides 130 to 4575, inclusive) and 5′ and 3′ untranslated regions of 129 and 220 nucleotides, respectively. The extreme 5′ and 3′ 19 and 20 nucleotides, respectively, are derived from primers designed from CFTR sequences of other species and may contain one or more nucleotide differences from the actual ovine sequence over these regions. The sequence context around the ATG translational start codon (positions 130–132) of the ovine CFTR cDNA is ACC**ATG**C and fulfills the Kozak consensus (34) with the invariant A at position −3, although the C at position +4 is not the usual G. A stop codon (TAG) is positioned at nucleotides 4573–4575.

Interspecies Homologies. A comparison at the nucleotide and predicted amino acid levels among CFTR sequences from sheep, human, cow, and mouse reveals that the sheep homologue of CFTR displays a very high level of sequence identity to the human CFTR cDNA. At the DNA coding level, the identity between sheep and human is 90.7% (compared with 80.7% between human and mouse). At the predicted amino acid level, the sheep and human CFTR polypeptides show 90.8% overall identity and 95.3% similarity when conservative amino acid differences are considered (compared with 77.7% and 88.7%, respectively, between human and mouse).

The homology between sheep and cow, which are more closely related species, is particularly striking (97.8% identical at the DNA coding level and 97.7% identical and 99.1% similar at the polypeptide level). Both sheep and cow CFTR genes code for polypeptides of equal length (1481 amino acids) and the 3-bp insertion/deletion with respect to cow and human CFTR (32) is reproduced in the sheep. Two "insertions" have been identified in the sheep CFTR with respect to the cow sequence: an extra three T residues in the 3′ untranslated region of the ovine CFTR sequence, part of a long run of T residues from 4660 to 4670; and an extra G in the 5′ untranslated leader sequence in sheep at position 106, which is also found in the human sequence.

A domain-specific comparison between human and sheep shows that nucleotide binding domains NBD1 and -2 and MSH1, -2, -5, -6, -8, -11, and -12 are more highly conserved than the regulatory (R) domain and MSH3, -4, -7, -9 and -10. Both NBDs show an amino acid identity of over 97% and a similarity of over 98%. Several of the 12 individual MSHs, which make up the two membrane-spanning domains of the CFTR protein, are very highly conserved: MSH1, -2, -5, -6, -8, -11, and -12 all show a 100% similarity with their human CFTR counterparts, reflecting data previously reported in a cross-species comparison of CFTR (32). Why certain MSHs should be more conserved than others is unknown, although it may imply differential functional importance of specific helices within each membrane-spanning domain.

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   1 MQRSPLEKAS VVSKLFFSWT RPILKKGYRQ RLELSDIYHI SSSDSADNLS
  51 EKLEREWDRE LASKKNPKLI NALRRCFFWR FMFYGIILYL GEVTKAVQPL
 101 LLGRIIASYD PDNKVERSIA IYLGIGLCLL FIVRTLLLHP AIFGLHHIGM
 151 QMRIAMFSLI YKKTLKLSSR VLDKISIGQL VSLLSNNLNK FDEGLALAHF
 201 VWIAPLQVTL LMGLLWDLLQ AFTFCGLAFL VVLALLQAGL GKMMMKYRDQ
 251 RAGKINERLV ITSEMIENIQ SVKAYCWEEA MEKIIENLRQ TELKLTRKAA
 301 YVRYLNSSAF FFSGFFVVFL SVLPYALLKG IILRKIFTTI SFCIVLRMAV
 351 TRQFPWAVQT WYDSLGAINK IQDFLQKQEY KTLEYNLTTT DVVMENVTAF
 401 WEEGFSKLFE KAKENNNNRK ISNCDTSLFF SNLLLGTPVL KDISFKIERG
 451 QLLAVAGSTG AGKTSLLMMI MGELEPSEGK IKHSGRISFC SQYSWIMPGT
 501 IKDNIIFGVS YDEYRYRSVI KACQLEEDIS KFSEKDNIVL GEGGITLSGG
 551 QRARISLARA VYKDADLYLL DSPFGYLDVL TEKEIFESCV CKLMANKTRI
 601 LVTSKMEHLK KADKILILHE GSVYFYGTFS ELQNQRPDFS SKLMGCDTFD
 651 QFTAERRNSI ITETLRRFSL EGDTSVSWNE TKKPSFKQTG EFGEKRKNSI
 701 LNSINSIRKF SVVQKTSLQM NGIDGASDEP LERRLSLVPH SEPGEGILPR
 751 SNAVNSGPTF LGGRRQSVLN LMTCSSVNQG QSIHRKTATS TRKMSLAPQA
 801 SLAEIDIYSR RLSQDTGLEI SEEINEEDLR DCFFDDVENI PAVTTWNTYL
 851 RYITVHKSLM FVLIWCLVVF LVEVAASLVV LCLFPKILLQ DKGNSTKNAS
 901 NSYAVIITST SSYYIFYIYV GVADTLLALG LFRGLPLVHT LITVSKTLHH
 951 KMLQSVLQAP MSTLNTLKTG GILNRFSKDI AVLDDLLPLT IFDFIQLLLI
1001 VIGAVVVVSV LQPYIFLATV PVIAAFILLR GYFLHTSQQL KQLESEGRSP
1051 IFTHLVTSLK GLWTLRAFGR QPYFETLFHK ALNLHTANWF LYLSTLRWFQ
1101 MRIEMIFVIF FIAVTFISIL TTGEGEGRVG IILTLAMNIM GTLQWAVNSS
1151 IDVDSLMRSV SRVFKFIDMP TEDGKPNNSF RPSKDSQPSK VMIIENQHVK
1201 KDDIWPSGGQ MTVKDLTAKY IDGGNAILEN ISFSISPGQR VGLLGRTGSG
1251 KSTLLLAFLR LLNTKGEIQI DGVSWDSITL QQWRKAFGVI PQKVFIFSGT
1301 FRKNLDPYEQ WSDQEIWKVA DEVGLRSVIE QFPGKLDFVL VDGGCVLSHG
1351 HKQLMCLARS VLSKAKILLL DEPSAHLDPI TYQIIRRTLK QAFADCTVIL
1401 SEHRIEAMLE CQRFLVIEEN KVRQYDSIQR MLSEKSLFRQ AISPADRLKL
1451 LPHRNSSRQR SRANIAALKE ETEEEVQETK L-
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FIG. 2. Predicted amino acid sequence of the ovine CFTR protein. The one-letter amino acid code is used throughout.

The R domain shows the lowest level of homology: the amino acid sequence identity between human and sheep is 83% (88.8% similarity), in agreement with the idea that the functional requirements for the R domain are not linked to absolutely conserved primary structure (32). Even so, within the R domain, short regions of conserved amino acid sequences are present and may represent important functional sites.

Expression of Ovine CFTR mRNA. To study the pattern of CFTR expression in the sheep, two different approaches were employed: RT-PCR and Northern analysis of total RNA.

RT-PCR. RT-PCR was used to selectively search total RNA from different fetal and adult sheep tissues for two independent gene transcripts. One set of primers would amplify the C fragment of the ovine CFTR message, while another set amplified the transcript encoding the c subunit of ovine ATP synthase (25) and acted as an internal control in the experiment. Detection of the CFTR and ATP synthase transcripts in different tissues at different developmental stages is shown in Fig. 3. Each lane shows the RT-PCR product from 1 μg of total RNA. The larger RT-PCR product (792 bp) is specific for CFTR and the smaller product (530 bp) represents the ATP synthase subunit c control housekeeping transcript. As it is a housekeeping gene, it is likely that the ATP synthase subunit c gene is expressed at equivalent levels in each tissue analyzed. In the adult sheep, CFTR is expressed at relatively high levels in duodenum, distal ileum, and liver; possibly at intermediate levels in the lung and colon; at lower levels in the trachea; and is not expressed in skeletal muscle. In late-fetal life, CFTR is highly expressed in duodenum, distal ileum, colon, pancreas, gall bladder, and liver; expressed at lower levels in lung and testis; expressed at very low levels in kidney, ovary, and thymus; and expressed at barely detectable levels in skeletal muscle. In midfetal life, CFTR is expressed at high levels in duodenum, cecum, colon, and gall bladder; at intermediate levels in trachea, lung, distal ileum, pancreas, liver, and thymus; at low levels in kidney, testis, and cardiac muscle; and at barely detectable levels in skeletal muscle.

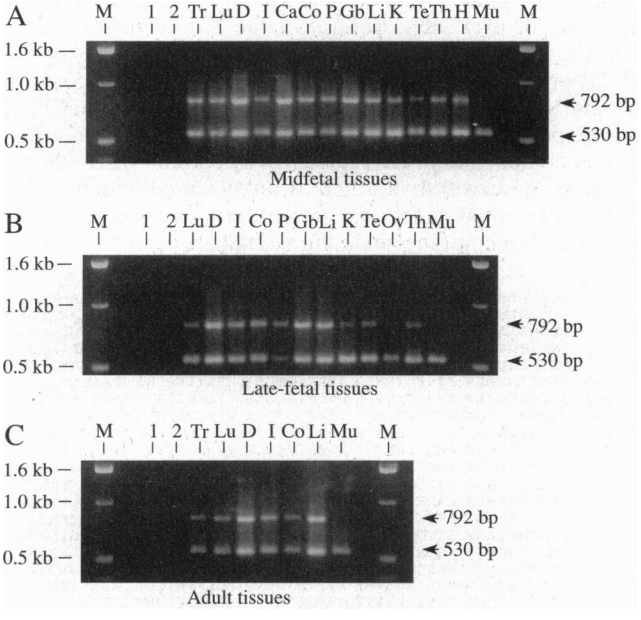

FIG. 3. RNA expression analysis by RT-PCR. Total RNA (1 μg) isolated from midfetal (*A*), late-fetal (*B*), or adult (*C*) sheep tissues was subjected to RT-PCR to coamplify the ovine CFTR fragment C (792 bp) and the ovine ATP synthase subunit c gene (530 bp). The latter was used as a constitutively expressed internal control. Lane M, 1-kb DNA ladder size markers (GIBCO/BRL); lane 1, no reverse transcriptase; lane 2, no RNA. Tr, trachea; Lu, lung; D, duodenum; I, distal ileum; Ca, cecum; Co, colon; P, pancreas; Gb, gall bladder; Li, liver; K, kidney; Te, testis; Ov, ovary; Th, thymus; H, heart; Mu, skeletal muscle.

Northern Analysis. Semiquantitative data on the level of CFTR expressed in different tissues at different stages of development were obtained by Northern analysis of total RNA samples from sheep tissue by using an ovine CFTR D′ fragment probe. Fig. 4 shows the results, including a representation of the relative loading levels of RNA for each sample, revealing CFTR to be expressed in a tissue-specific and developmental-specific manner.

Table 2 contains a summary of the Northern analysis data from the different tissues analyzed, showing the levels of CFTR expression relative to the total amount of RNA in each lane. It is clear that CFTR is expressed at relatively high levels during midgestation fetal life, especially in the duodenum, distal ileum, cecum, colon, pancreas, and gall bladder, while being expressed at lower levels in the lung. CFTR expression in the late-fetal lung is at a level equivalent to that observed in the midfetal lung, but transcripts are not seen in adult lung tissue. Interestingly, although high levels of CFTR expression could be seen in fetal colon, no detectable expression was found in adult colon. No expression could be seen in the fetal kidney, although RT-PCR results have shown CFTR transcripts to be present in this organ, though probably at levels too low to be detected by Northern analysis. Low levels of CFTR transcripts were identified in midfetal, late-fetal, and adult liver tissue. Despite very low levels of CFTR being detected in fetal skeletal muscle by RT-PCR, no detectable CFTR expression was found in skeletal muscle at any of the sheep developmental stages analyzed by Northern analysis.

CFTR has previously been shown to be expressed in humans at the apical surface of certain epithelial cells (35–37), most noticeably in cells of the submucosal glands of the airway, in

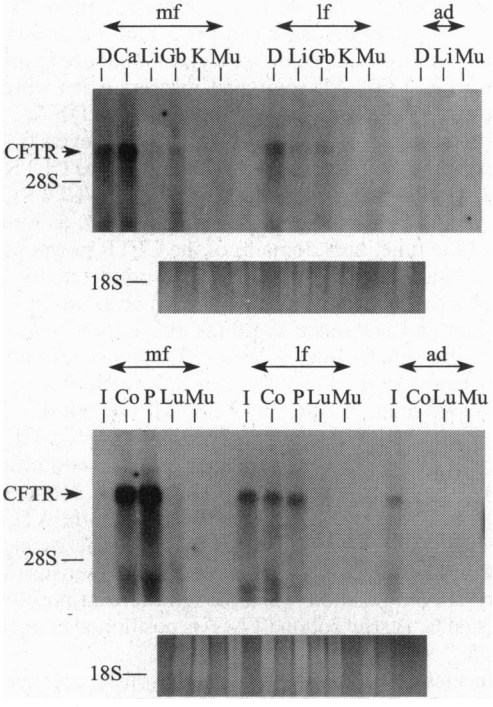

FIG. 4. Spatial and temporal expression of CFTR in sheep. Northern blots of total RNA (≈20 μg) from different tissues (fetal and adult) hybridized with ovine CFTR cDNA fragment D′ (^{32}P-labeled to a specific activity of 2–4 × 10^8 cpm/μg). The panels directly below the autoradiograms show representations of the RNA loading for each sample. The positions of 28S and 18S ribosomal RNA bands are indicated. Tissue abbreviations as in the legend to Fig. 3. mf, Mid-fetal; lf, late fetal; ad, adult.

Table 2. Summary of CFTR expression in fetal and adult sheep tissues

Tissue	Relative CFTR expression		
	Midfetus*	Late fetus†	Adult female‡
Lung	++	++	−
Duodenum	+++	+++	++
Distal ileum	+++	+++	+++
Cecum	+++	ND	ND
Colon	+++	+++	−
Pancreas	+++	+++	ND
Gall bladder	+++	++	ND
Liver	+	+	+
Kidney	−	−	ND
Skeletal muscle	−	−	−

Levels of total RNA in each lane of the Northern blot were standardized and relative levels of CFTR expression were determined. −, No detectable expression; +, very low levels of expression; ++, medium levels of expression; +++, highest levels of expression. ND, not determined.
*Gestational day 70 (equivalent to 18-week human fetus).
†Gestational day 138 (10 days from birth).
‡Pregnant ewe (midterm; gestational day 70).

pancreatic ducts, bile ducts, crypts of the small intestine, colon, and sweat gland ducts. The expression of CFTR in adult sheep is consistent with the pattern seen in adult humans. CFTR expression in human fetuses occurs as early as 12 weeks in epithelia of the lung, pancreas, small intestine, colon, and male genital ducts (18, 19); however, expression in the newborn and adult human respiratory epithelium is at very low levels compared to that in the fetal lung (19, 38). Likewise, our data indicate much greater CFTR expression in the lungs of fetal sheep than of adult sheep. Moreover, expression of CFTR in fetal sheep lung and gastrointestinal tracts is at high levels at least by 70 days of the 148-day gestation period, a point equivalent to an 18-week human fetus.

In summary, we have shown that the spatial and developmental expression of CFTR in the sheep is similar to that seen in the human. Therefore, the sheep lung may provide a useful system in which to reproduce CF lung disease. The cloning and subsequent sequencing of the ovine CFTR cDNA has provided the necessary information to embark on a genetic screening program to look for natural CFTR variants in sheep populations. Several putative polymorphisms have been identified thus far. To investigate the lung pathology in CF further, natural variants of sheep with CFTR mutations that lead to CF can be selected and bred to produce a line of animals with a CF lung disease phenotype.

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