Journal of Cell Science 107, 413-424 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994
JCS8468

Developmental expression of mucin genes *MUC1* and *MUC2*

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SUMMARY

The mucin gene *MUC1*, is expressed in a number of human ductal epithelia in vivo including those within the pancreas, mammary gland, kidney and genital ducts. Further it is expressed at a high level in certain tumours and tumour-derived cell lines. *MUC2* was initially isolated from a human jejunum cDNA library and is thought to be one of the major intestinal mucin genes, though it is also expressed in the trachea. We have examined the developmental expression of these two mucin genes in human tissues. High level expression of *MUC1* has been seen by 12.5 weeks of gestation in the epithelia of the distal respiratory tract and the collecting ducts in the kidney. By 18 weeks *MUC1* mRNA is detectable in the colon but pancreatic expression of *MUC1* is not seen until late in gestation. *MUC2* mRNA is seen by 12 weeks of gestation in the jejunum, ileum and colon, and in large bronchioles of the lung by 18 weeks. The pattern of expression of *MUC1* suggests that this mucin may not be involved in early ductal obstruction in the CF pancreas, but both *MUC1* and *MUC2* may play a role in the development of intestinal disease and *MUC1* in early respiratory disease associated with CF.

Key words: mucin, *MUC1*, *MUC2*, expression, human development

INTRODUCTION

Mucous glycoproteins, mucins, contain a high percentage of serine, threonine, proline, alanine and glycine in addition to a large quantity of complex O-linked oligosaccharides. The family of genes that encode the mucins expressed by most epithelia contains at least six and possibly more distinct genes *MUC1-MUC6*. There is very little sequence homology among the different types of mucin gene sequences (*MUC1-MUC6*) described to date, although there is substantial conservation in sequence among the same type of mucin (e.g. *MUC1*) in different species. There is one common feature of almost all mucin genes, which has come to be considered a motif for mucin, the tandem repeat. Mucin tandem repeats are regions of primary sequence (nucleic acid and amino acid) that contain several tandemly arrayed identical (or highly similar) repeats of shorter sequence elements. Mucin tandem repeat amino acid sequences generally contain a high percentage of serine and threonine residues that are the predominant sites of O-linked glycosylation.

The expression of specific mucins in tissues in vivo and in cells in culture has been well documented with a battery of anti-mucin monoclonal antibodies. Many of these antibodies were initially raised against malignant secretory epithelial cells and hence were classed as cancer-associated antigens. One significant problem with antibody studies of the tissue-specific expression patterns of mucin is the fact that many tissues attach different carbohydrate structures to the same mucin core proteins (Lan et al., 1990; Bara et al., 1993), while some tissues attach the same carbohydrate structure to different mucin core proteins (Baeckstrom et al., 1991). These carbohydrate structures can inhibit the binding of antibodies to some mucin core protein epitopes (Lan et al., 1990; Bara et al., 1993). Hence, when using antibodies to determine mucin expression, negative results should be carefully interpreted. The technique of in situ hybridization for the detection of mRNA, which is used in the studies presented here, circumvents this problem.

The best characterized of the mucin genes is *MUC1*. This was cloned independently from a human mammary tumour cell line (Gendler et al., 1987, 1990) and from a human pancreatic adenocarcinoma cell line (Lan et al., 1990). *MUC1* was initially defined as the major pancreatic/mammary gland mucin, while *MUC2* (Gum et al., 1989, 1992; Jany et al., 1991), *MUC3* (Gum et al., 1990), *MUC5* (Porchet et al., 1991) and *MUC6* (Tonibara et al., 1993) were proposed to be predominantly intestinal mucins. *MUC4* (Porchet et al., 1991) was suggested to be primarily a tracheobronchial mucin. There is, however, still some confusion as to the precise cell-specific localization of expression of the mucin genes in different organs in vivo. Further, it is not clear whether differentiated cell types within a given organ produce more than one mucin either simultaneously or at different developmental stages. These questions are of considerable importance in our understanding of mucin biology and further are relevant to the pathogenesis of human diseases such as cancer and cystic fibrosis.

In cystic fibrosis (CF) the major pathological problems are the result of an accumulation of mucins within the respiratory and digestive systems. Clearly these mucin deposits are likely

to be made up of a mixture of different mucin gene products that will depend in part on the specific tissue involved. In addition there are a number of reports of altered mucin biochemistry in CF, including increased sulphation and fucosylation, and decreased sialylation (Boat et al., 1974; Wesley et al., 1983; Scanlin et al., 1985; Rose, 1988). However, some of these experiments were performed on cell types, such as skin fibroblasts, that do not express the cystic fibrosis transmembrane conductance regulator (*CFTR*) and hence their relevance to CF is uncertain. The *CFTR* gene is known to code for a low conductance chloride ion channel that is regulated by cAMP (Rommens et al., 1989; Riordan et al., 1989; Gray et al., 1989; Anderson et al., 1991; Kartner et al., 1991; Bear et al., 1992). It remains unclear exactly how mutations in the *CFTR* gene result in the problems of mucus clearance that are associated with CF. In addition it is not yet known which mucin gene products are involved in the different tissues that are affected. Some of the side-effects of mucin accumulation are already apparent in foetuses with CF during the mid-trimester of development (Harris et al., 1991). The availability of cloned full-length cDNAs for several mucin genes will facilitate the direct examination of individual mucin glycoproteins that are affected in appropriate CF cell culture systems. In order to design a relevant approach to these experiments, it is essential to specifically identify which mucin genes are expressed in each of the epithelia that are involved in the pathology of CF and establish at what developmental stage that expression commences. The developmental expression of *MUC1* alone has been examined in the mouse (Braga et al., 1992). However, as there are some differences in the expression of *CFTR* in humans and mice (A. E. O. Trezise, unpublished observations), the current investigation of human development is warranted.

We have previously examined the expression of *CFTR* in human foetal tissues, by RNA in situ hybridization, in order to establish the age of onset of the disease process and to elucidate which tissues may be involved in early pathogenesis of CF (Foulkes and Harris 1993; Trezise et al., 1993). *CFTR* mRNA was shown to be expressed at a high level in mid-trimester pancreatic duct, in the crypts of the ileum and colon, in the bile ducts and at lower levels in genital ducts. Surprisingly, given the relatively low levels of *CFTR* mRNA in the respiratory epithelium after birth (Crawford et al., 1991), abundant expression of *CFTR* mRNA was seen throughout the lung epithelium during the mid-trimester (Trezise et al., 1993). It is possible that this reflects the presence of serous cells in the surface epithelium of the lung before but not after birth (Jeffrey and Reid 1977; McDowell et al., 1978). Later in life *CFTR* mRNA and protein appears largely restricted to the serous portion of submucosal glands (Engelhardt et al., 1993). In this report we examine the expression of *MUC1* and *MUC2* between the early mid-trimester of gestation and birth in order to look for possible involvement of these mucins in the early CF disease process.

MATERIALS AND METHODS

Materials

Tissues from mid-trimester terminations were obtained with local ethical committee approval. Foetuses examined were aged from 12 to 24 weeks after last menstruation, based on foot length. In addition tissues were examined from a 35-week spontaneous termination and three full-term neonatal deaths. Normal jejunum from the edge of tissue manifesting necrotizing enterocolitis was obtained from a 1-month baby undergoing surgery. Normal transverse colon tissue was obtained from a 7-month baby undergoing colostomy above an area of Hirschprung's disease. Normal adult lung tissue was collected during removal of lung tumours. Tissues for in situ hybridization were fixed directly in 4% paraformaldehyde, 100 mM NaOH, 100 mM $Na_2B_4O_7$, 10% sucrose, and frozen in liquid nitrogen prior to cutting 10 μm sections.

Methods

In situ hybridization was carried out essentially as described previously (Trezise et al., 1993). Two sets of probes were utilized for in situ localization of *MUC1* mRNA: the first, MUC1-1/2, corresponded to a 450 bp fragment from the tandem repeat of the *MUC1* cDNA (approximately 22 repeats of the sequence seen in base pairs 481-540, as in Fig. 1 of Lan et al., 1990); the second probe (MUC1-3/4) was a 345 bp fragment from a position 5′ of the tandem repeat portion of the *MUC1* cDNA (base pairs 1-345, as in Fig. 1 of Lan et al., 1990). Both probes were cloned into the Bluescript KS+ vector (Stratagene) and T7 and T3 RNA polymerase promoters in the vector were used for the generation of radiolabelled antisense or sense probes incorporating ^{35}S-UTP. The MUC1-1 and -3 probes were synthesized in the antisense direction and the MUC1-2 and -4 probes were the corresponding probes synthesized in the sense direction. The *MUC2* probe pHAM 1, a 90 bp portion of the *MUC2* tandem repeat inserted at the *Eco*RI site of Bluescript SK–, isolated from a tracheal cDNA library (Jany et al., 1991), was provided by Dr C. Basbaum. MUC2-1 was generated in the antisense direction and MUC2-2 in the sense direction. Two sets of probes were utilized for in situ localization of *CFTR* mRNA: the first, HCF-1/2, corresponded to a *Ssp*I/*Hpa*I fragment of the R domain (exon 13, base pairs 1977-2461 of the cDNA); the second, HCF-3/4, was an *Eco*RI/*Xba*I fragment (exons 1-5, base pairs 62-645) of the 10-1 *CFTR* cDNA; both were cloned into the Bluescript KS+ vector (Stratagene) and T7 and T3 RNA polymerase promoters in the vector were used for the generation of radiolabelled antisense or sense probes incorporating ^{35}S-UTP. The HCF-1 and HCF-3 probes were synthesized in the antisense direction and the HCF-2 and HCF-4 probes were the corresponding probes synthesized in the sense direction. Tissue sections were digested with proteinase K prior to 18 hour hybridization with 1×10^7 cpm/ml of ^{35}S-labelled RNA probe in 50% formamide at 55-60°C. Sections were then digested with RNase A and washed at a stringency of 0.1× SSC at 60°C for CFTR probes and at 70°C for MUC1 and MUC2 probes. Slides were dipped in Kodak NTB-2 or Amersham LM1 emulsion, exposed for 2 weeks at 4°C, developed and counterstained with haematoxylin and eosin.

Multiple tissue sections from different foetuses and neonatal or adult tissues were hybridized with each probe set and in all cases results were consistent.

RESULTS

The results of in situ hybridization experiments with *MUC1* are shown in Figs 1,2,4-7 and summarized in Table 1. Results with *CFTR* are shown in Figs 1,2,4 and 6. *MUC2* results are shown in Fig. 3 and summarized in Table 2.

Respiratory system

MUC1 expression is detected by 12.5 weeks of gestation within the epithelium of the respiratory system. The pattern and levels of expression of *MUC1* mRNA remained similar from this age until late in gestation as illustrated in Fig. 1 (A-C)

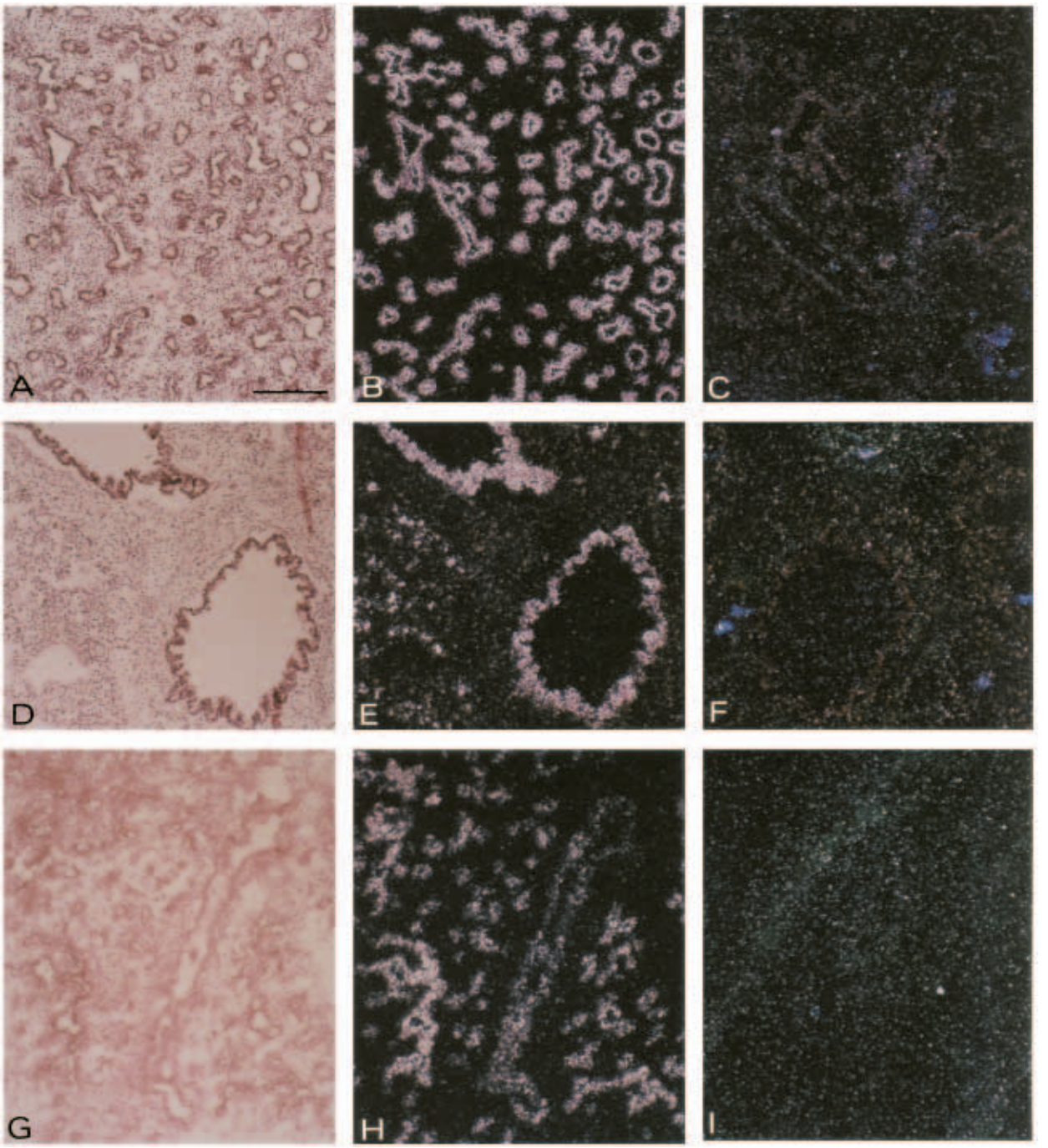

Fig. 1. Expression of *MUC1* and *CFTR* in 18.5-week and term foetal lung. Expression of *MUC1* mRNA (A-C) and *CFTR* (G-I) in the immature (18.5-week) bronchiole, smaller airways and terminal sacs and of *MUC1* in term lung (D-F). (A and D) Brightfield views of sections hybridized with the MUC1-3 (antisense) probe. (B and E) Darkfield images of the respective sections. (C and F) Darkfield views of consecutive sections hybridized with the MUC1-4 (sense) negative control probe. (G) A brightfield view of a section of the same (18.5-week) foetal lung shown in (A-C) hybridized with the HCF3 (antisense) probe. (H) A darkfield image of the same section. (I) A darkfield view of a consecutive section hybridized with the HCF4 (sense) negative control probe. Bar, 200 μm.

in an 18.5-week fetal lung. *MUC1* expression was seen in the epithelium lining the small bronchi, bronchioles and in some fetal lungs at slightly lower levels in the more distal parts of the developing airway. This pattern of expression is in contrast to that seen for *CFTR* mRNA in the mid-trimester lung where the expression levels rise substantially moving from bronchioles to more distal parts of the conducting airway (Fig. 1G-I). In summary, *MUC1* is expressed at high levels throughout the developing airway epithelium often with a slight decrease of expression moving from the bronchi through to the terminal sacs, while *CFTR* shows an definite opposite gradient with maximal expression in the terminal sacs. *MUC1*

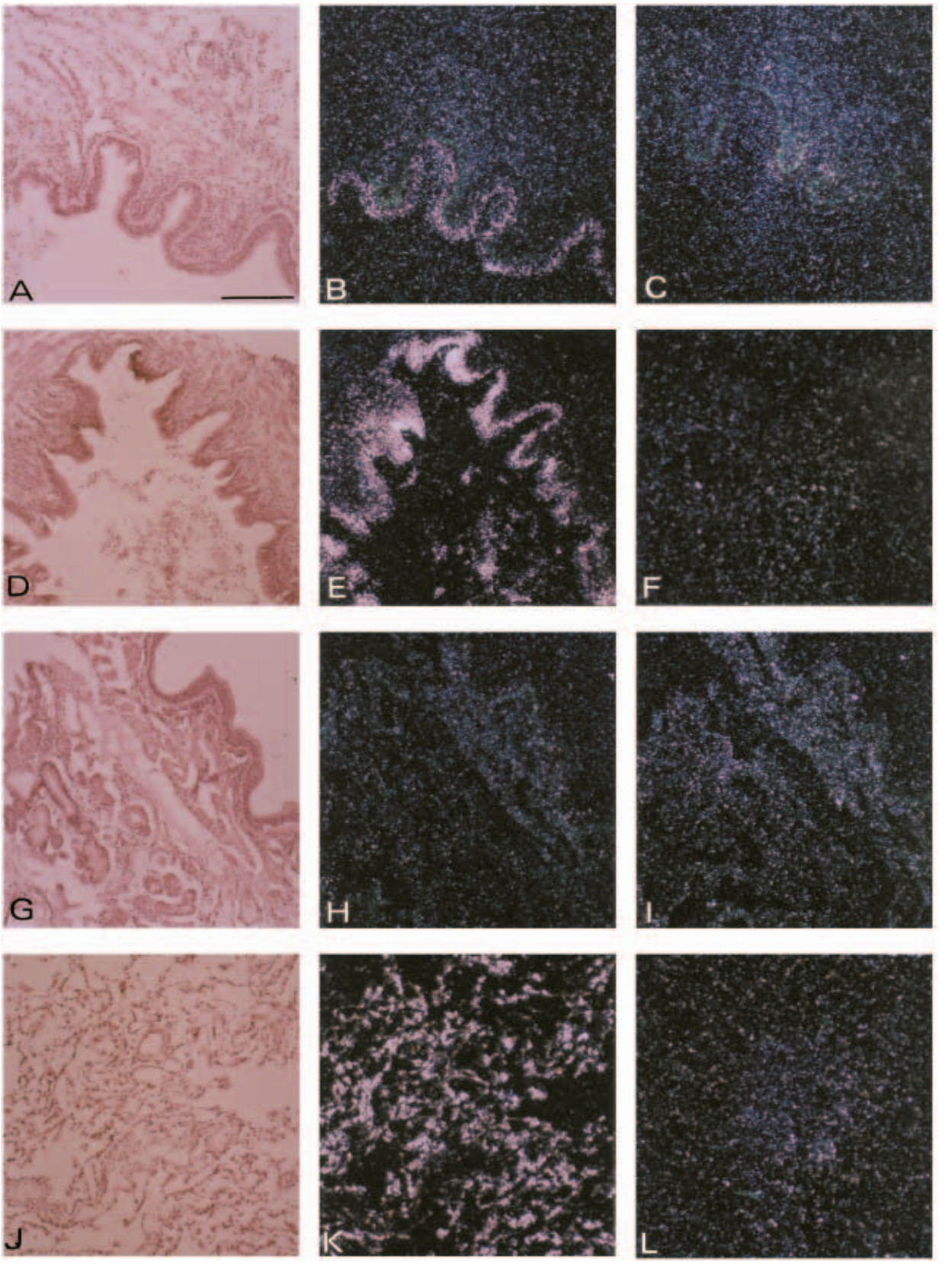

Fig. 2

Fig. 2. Expression of *MUC1* and *CFTR* in adult lung. Expression of *MUC1* mRNA (A-F and J-L) and *CFTR* (G-I) in bronchus, bronchioles and smaller airways of an adult lung. (A) Bronchus, (D) bronchiole, and (J) smaller airways, show brightfield views of sections hybridized with the MUC1-3 (antisense) probe. (B) (E) and (K), respectively, show darkfield images of the same sections. (C,F and L) Darkfield views of consecutive section hybridized with the MUC1-4 (sense) negative control probe. (G) Bronchiole, shows a brightfield view of a section hybridized with the HCF3 (antisense) probe. (H) Darkfield image of the same section. (I) A darkfield view of a consecutive section hybridized with the HCF-4 (sense) negative control probe. Bar, 200 μm.

expression in adult lung was seen at low levels in the epithelium of the main bronchus (Fig. 2A-C) and at higher levels in the bronchiolar epithelium (Fig. 2D-F) and in terminal sacs (Fig. 2J-L). In equivalent sections of adult bronchus, bronchiole (Fig. 2G-I) and terminal sacs no *CFTR* mRNA was detected.

Expression of *MUC2* in the developing respiratory epithelium was restricted to larger bronchioles relatively late in development (after 19 weeks) and levels of *MUC2* mRNA were very low (not shown). In adult lung *MUC2* was clearly seen, at higher levels than are observed in 19-week foetuses, in goblet cells in the epithelium of the main bronchus and of bronchioles (Fig. 3I,J).

Digestive system

Intestine

Expression of *MUC1* in the gut was first detected at week 16 of gestation with *MUC1* mRNA being restricted to the epithelium of the colon. The expressing cells are located largely within the crypts of the epithelium, with levels decreasing as the cells migrate along the villi. This pattern of expression remains constant throughout foetal development (Fig. 4A-E) though the relative levels of *MUC1* expression in the colon appear to increase through gestation (Fig. 5A-C). No expression of *MUC1* was seen elsewhere in the gut during the mid-trimester. The localization of expression of *MUC1* mRNA in the colon at 7 months after birth is slightly different from that seen in foetal life, with a reduction in the abundance of *MUC1* mRNA in colonic epithelium (Fig. 5D,E).

Like *MUC1*, *CFTR* expression in the foetal colon is largely restricted to the crypt cells (Fig. 4F-H); however, *CFTR* mRNA is also seen in the crypts of the small intestine.

MUC2 expression is detected by 12 weeks gestation in the jejunum, ileum and colon. The pattern of expression is distinct from that seen for *MUC1*. Rather than being evident solely in crypt cells, *MUC2* is expressed at high levels by individual cells (probably goblet cells) throughout the intestinal epithelium, as evidenced by a punctate pattern of silver grains (Fig. 3A-H). However, throughout the jejunum (Fig. 3A), ileum (Fig. 3B,C) and colon (Fig. 3D-H) the concentration of cells expressing *MUC2* is higher within the crypt than along the villus epithelium.

Table 1. Expression of *MUC1*

	Tissue					
			Intestine			
Age	Pancreas	Lung	Jej	Ile	Col	Kidney
12.5 w	ND	+	–	ND	ND	+
14 w	–	+	–	–	–	+
16 w	–	+	–	–	+/–	+
19 w	–	+	–	–	+	+
Term	+	+	–	–	+	+
7 month	ND	ND	ND	ND	+	ND
Adult	ND	+	ND	ND	ND	ND

Tables 1 and 2. Jej, jejunum; Ile, ileum; Col, colon; w, weeks. ND, not done.

Table 2. Expression of *MUC2*

	Tissue					
			Intestine			
Age	Pancreas	Lung	Jej	Ile	Col	Kidney
12 w	–	–	+	+	+	–
14 w	–	–	+	+	+	–
16 w	–	–	+	+	+	–
19 w	–	+	+	+	+	–
Term	–	+	+	+	+	–
1 month	ND	ND	+	ND	ND	ND
7 month	ND	ND	ND	ND	+	ND
Adult	ND	+	ND	ND	ND	ND

Pancreas

Significant expression of *MUC1* in the pancreas was not detected until term when *MUC1* mRNA was seen in the epithelium of large intralobular pancreatic ducts (Fig. 6A-C). This is in contrast to the expression of *CFTR* mRNA, which was seen to be abundant in the ductal epithelium of mid-trimester pancreas (Fig. 6G-I). Consecutive sections of mid-trimester pancreatic tissue were probed for *CFTR* and *MUC1* mRNA expression and were constistently positive and negative, respectively.

MUC2 mRNA was not detected within the pancreas at any gestational age.

Urinogenital tract

MUC1 mRNA was detected in kidney at 12 weeks of gestation and showed a constant pattern of expression throughout the mid-trimester (Fig. 7). *MUC1* mRNA was abundant in the epithelium of the collecting tubules of the cortex and the collecting ducts of the medulla. Neither *CFTR* expression nor *MUC2* mRNA were detected in the mid-trimester kidney or in the term kidney (data not shown). Expression of *MUC1* and *MUC2* in the male genital ducts was very low at all ages examined (data not shown).

DISCUSSION

Obstruction of pancreatic ducts and the intestine by mucous secretions are early features of the pathology of cystic fibrosis and may be present by the mid-trimester of development (Harris et al., 1991). Clearly these mucous secretions are likely to include the products of several different mucin genes. We have examined the developmental expression of two mucin genes, *MUC1* and *MUC2*, in order to establish their potential involvement in this disease process. We have shown previously (Foulkes et al., 1993; Trezise et al., 1993) that the *CFTR* gene is expressed early in the mid-trimester of human development, specifically in the epithelium of pancreatic ducts, proximal and distal airways, in the crypt cells of the small intestine and colon, and in male genital ducts. This suggests

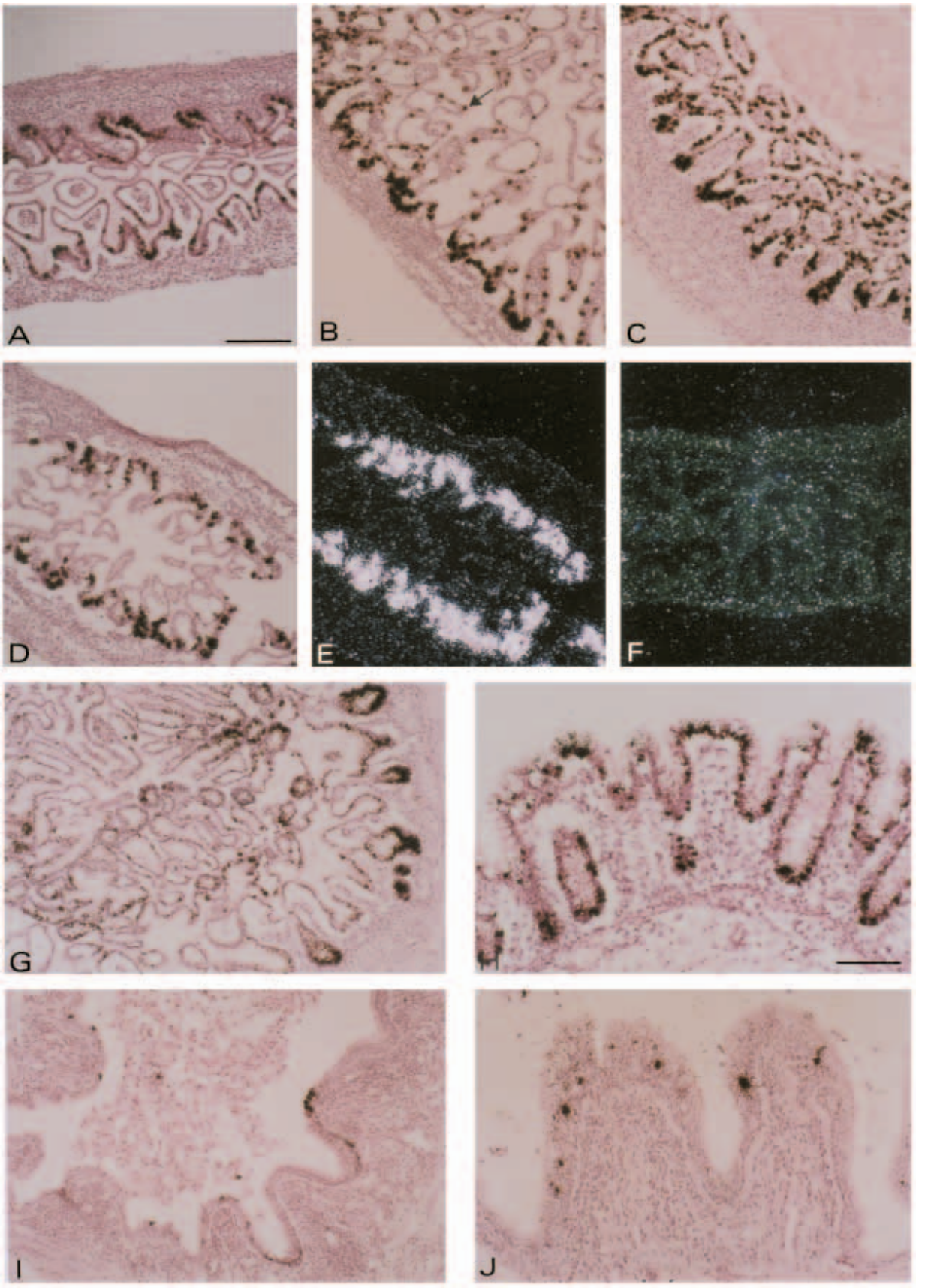

Fig. 3. For legend see p. 420

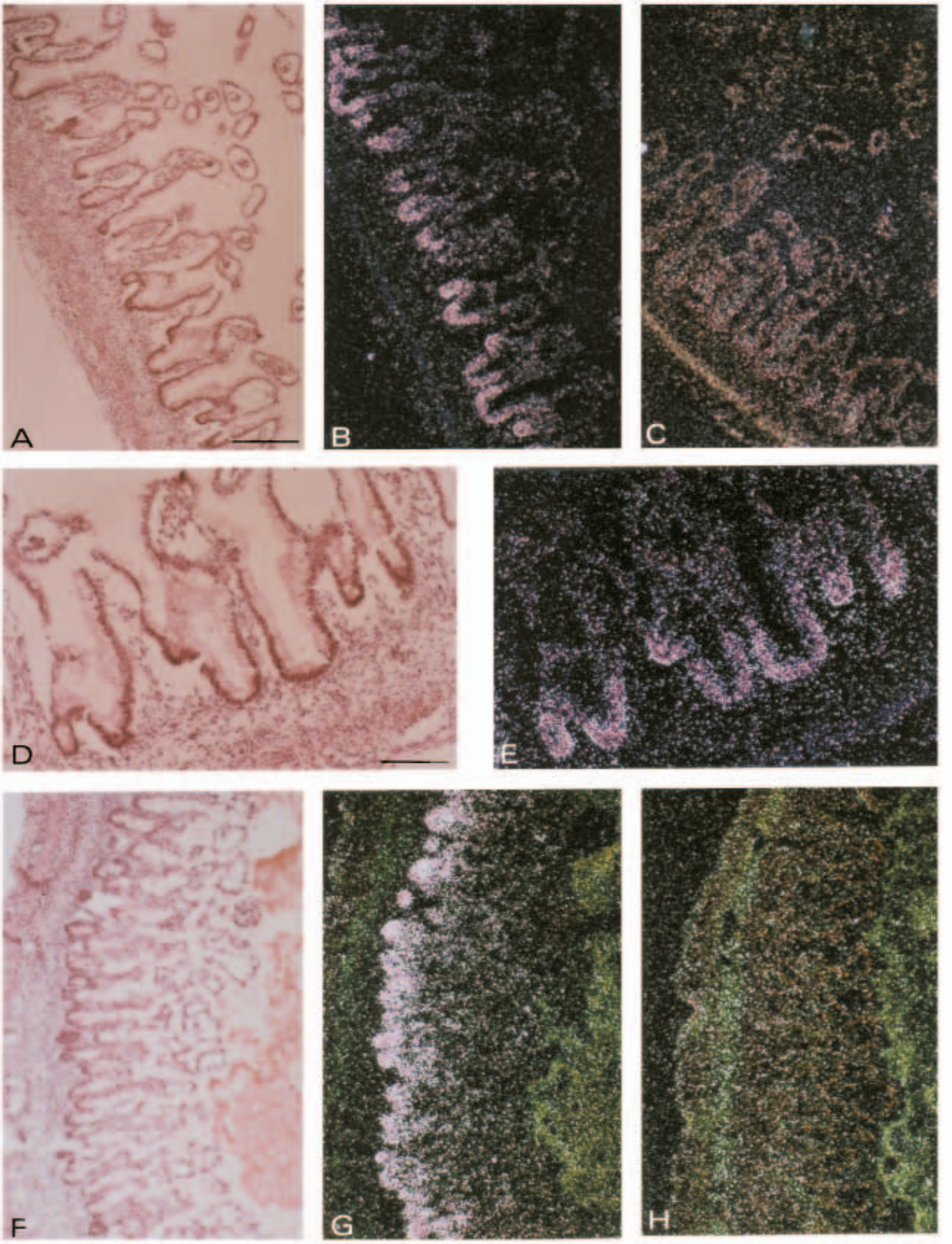

Fig. 4. For legend see p. 420

Fig. 3. Expression of *MUC2* in foetal intestine and adult lung. (A, B and C) Brightfield views of sections from 14-week foetal jejunum, 13-week ileum and 19-week ileum, respectively, hybridized with the MUC2-1 (antisense) probe. The black dots clearly show high level expression of *MUC2* mRNA. (D,E and F) *MUC2* expression in the 13-week foetal colon. (D) Brightfield and (E) darkfield image of the same section hybridized with the MUC2-1 probe. (F) Darkfield view of an adjacent section hybridized with the MUC2-2 (sense) probe. (G and H) Brightfield views of 19.5-week foetal colon and term colon, respectively, sections hybridized with the MUC2-1 probe. (I) *MUC2* expression in the adult bronchus. (J) *MUC2* expression in an adult bronchiole. Bars: (A-G and I), 200 μm; (H and J), 100 μm.

Fig. 4. Expression of *MUC1* and *CFTR* in 18- to 19-week foetal colon. (A) A brightfield view of a section from 19-week colon hybridized with the MUC1-3 (antisense) probe. (B) A darkfield image of the same section. (C) A darkfield view of a consecutive section hybridized with the MUC1-4 (sense) negative control probe. (D) Brightfield, and (E) darkfield, show a higher magnification view of the colonic crypts, the white dots clearly show high level expression of *MUC1* mRNA. (F) Brightfield view of a section of 18-week colon hybridized with the HCF3 (antisense) probe; and (G) a darkfield image of the same section. (H) A darkfield view of a consecutive section hybridized with the HCF4 (sense) negative control probe. Bars: (A-C and F-H), 200 μm; (D and E), 100 μm.

that the disease process has commenced by early mid-trimester. We now show that *MUC1* and *MUC2* have highly restricted patterns of expression in epithelial cells during development.

Respiratory system

MUC1 transcription is evident by 12.5 weeks of gestation throughout the airway epithelium from bronchi to developing distal end sacs. The levels of *MUC1* RNA in the airway epithelium often show a slight gradient of expression, with highest levels being detected in the larger airways and lower levels in distal airways. These patterns of expression remain constant through to term. Within the adult lung *MUC1* is expressed at low levels within the epithelium of the bronchi and at higher levels in bronchioles and in terminal sacs. This is in marked contrast to the expression of *CFTR*, which, though it is evident in the respiratory epithelium during early development, with highest levels being detected in the most distal portions of the airways, is no longer seen in this epithelium after birth.

Postnatally *CFTR* expression in the lung appears restricted to certain epithelial cells within the serous portion of submucosal glands (Englehardt et al., 1993), which is an enigma, since the lung is clearly important in the pathology of CF postnatally. There are some reports of distension of submucosal

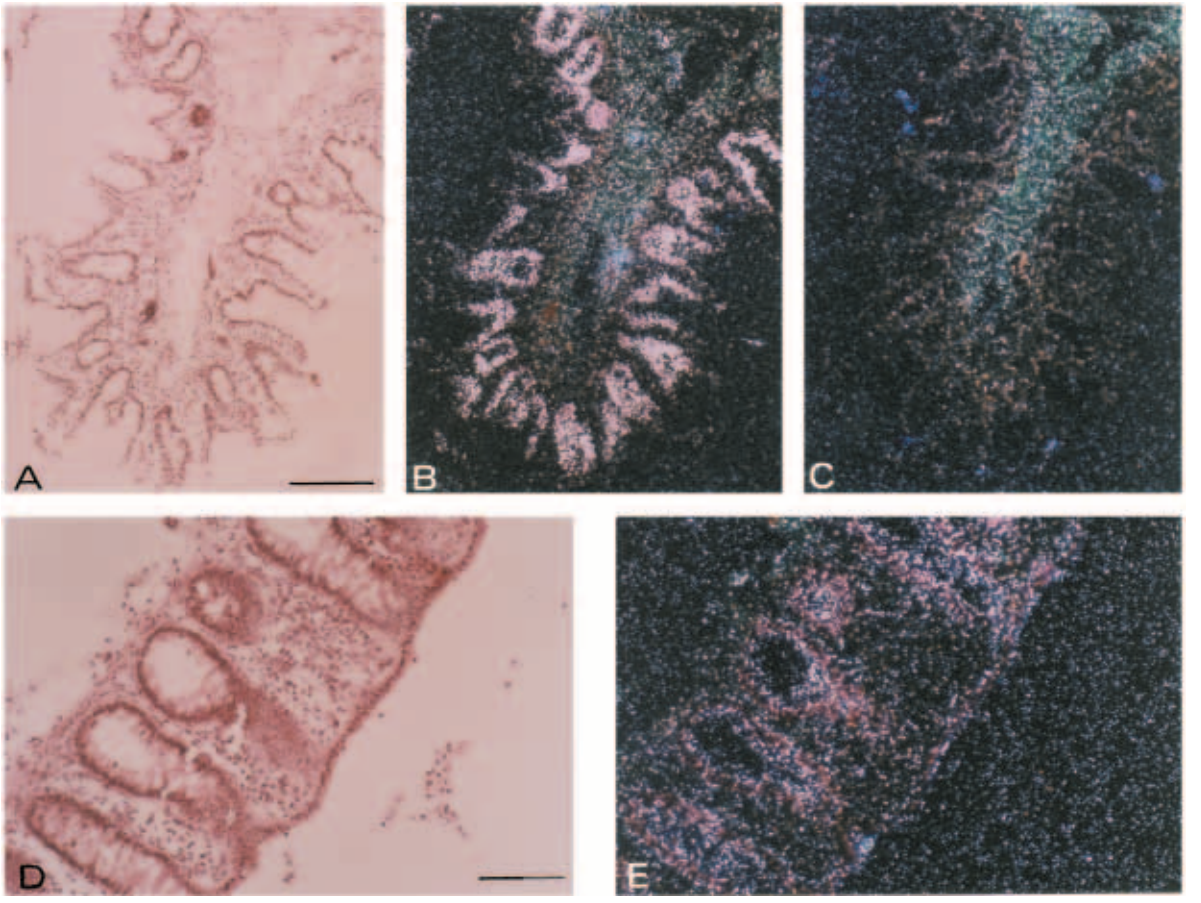

Fig. 5. Expression of *MUC1* in colon at term and at 7 months. (A-C) *MUC1* expression at term. (A) A brightfield view of a section hybridized with the MUC1-3 (antisense) probe. (B) A darkfield image of the same section. (C) A darkfield view of a consecutive section hybridized with the MUC1-4 (sense) negative control probe. (D) Brightfield and (E) darkfield, show expression of *MUC1* (as shown by MUC1-3) in 7-month colon. Bars: (A,B and C), 200 μm; (D and E), 100 μm.

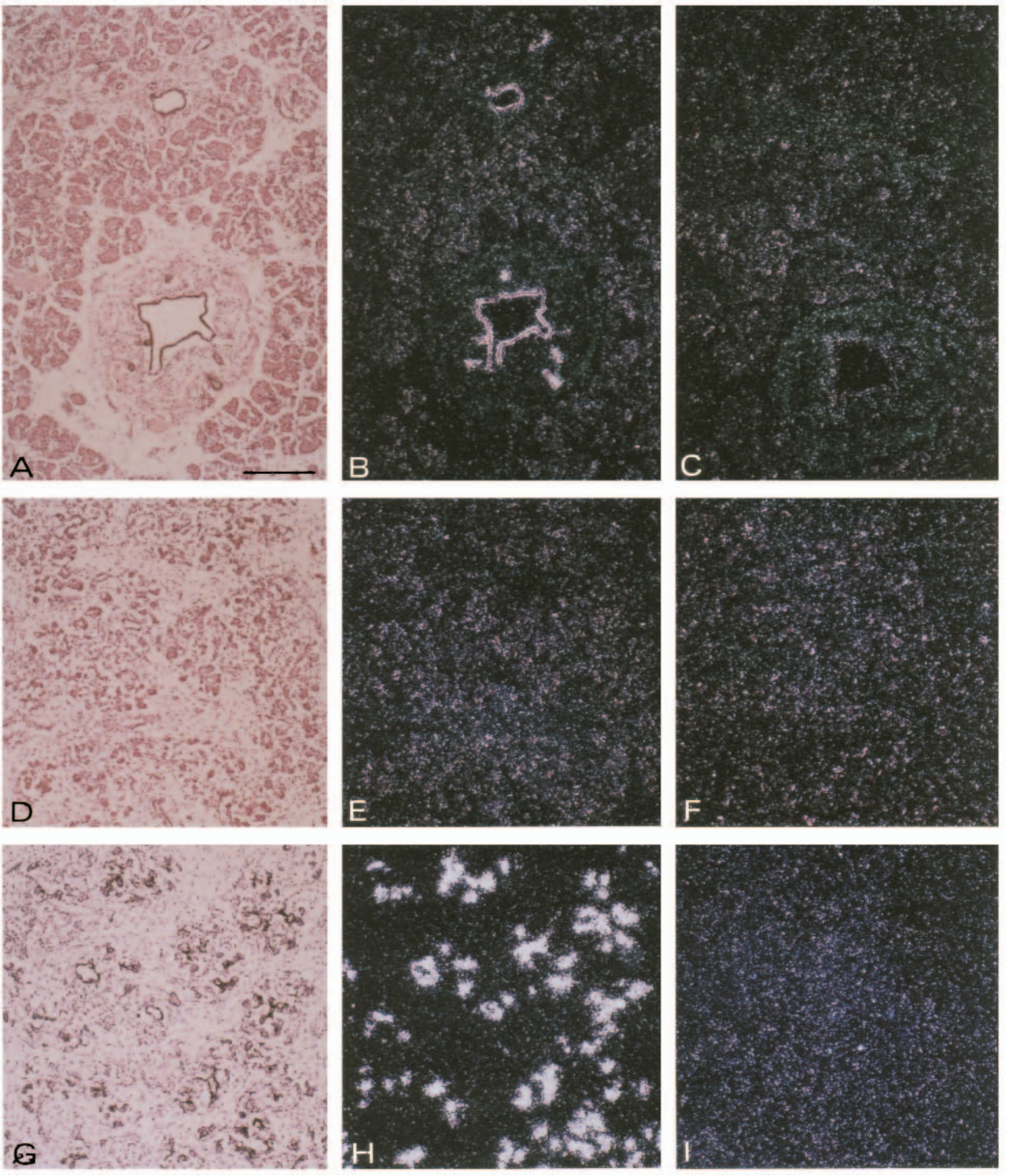

Fig. 6. Expression of *MUC1* and *CFTR* in the 18.5-week and the term pancreas. Expression of *MUC1* mRNA in term interlobular ducts (A-C). (A) A brightfield view of a section hybridized with the MUC1-1 (antisense) probe. (B) A darkfield image of the same section. (C) A darkfield view of a consecutive section hybridized with the *MUC1-2* (sense) negative control probe. (D-I) Comparative expression of *MUC1* (D-F) and *CFTR* (G-I) in the same 18.5-week foetal pancreas. No expression of *MUC1* is seen in the 18.5-week foetal pancreas as is shown by a lack of binding of MUC1-1 in brightfield (D) or darkfield (E) image. The negative control probe MUC1-2 is shown in (F). In the same 18.5-week pancreas high levels of *CFTR* mRNA are detected with the HCF-3 antisense probe in the epithelium lining intralobular and interlobular ducts (G, brightfield; H, darkfield). The negative control HCF-4 sense probe is seen in (I). Bar, 200 μm.

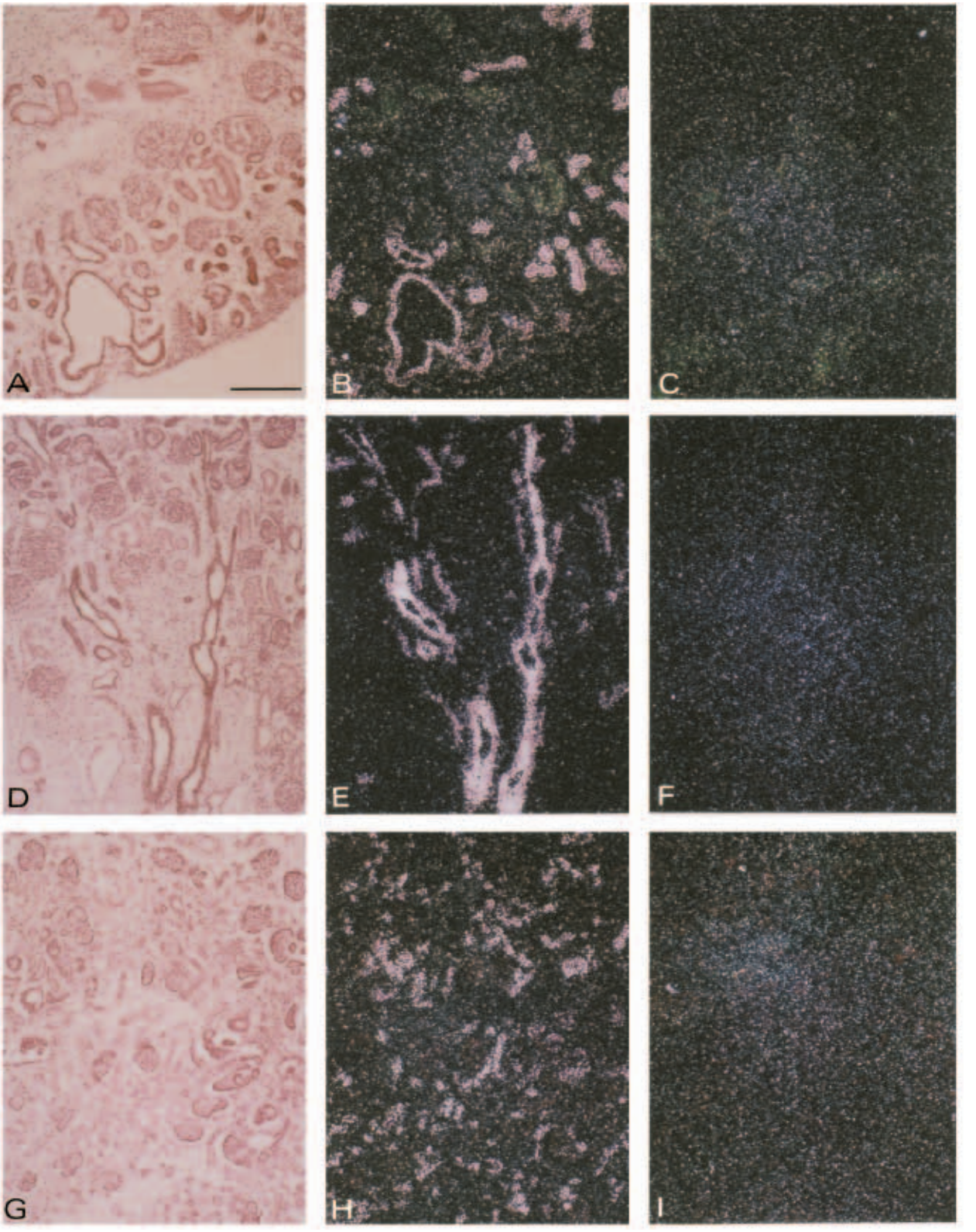

Fig. 7. Expression of *MUC1* in 12.5-, 18.5-week and term foetal kidney. (A-C) Sections through a 12.5-week kidney; (D-F) a 18.5-week kidney; (G-I) a term (1.5 hour) kidney. (A,D and G) Brightfield views of kidney sections hybridized with the MUC1-3 (antisense) probe. (B,E and H) Darkfield images of the same sections. (C,F and I) Darkfield views of consecutive sections hybridized with the MUC1-4 (sense) negative control probe. Bar, 200 μm.

glands in the CF lung perinatally. Other reports suggest that lung anatomy is normal until after the first pathogenic infection. In either case, the presence of *MUC1* mucin within the repiratory epithelium throughout gestation suggests the possibility that it may contribute to CF airway disease.

Very low levels of *MUC2* expression are detected in the respiratory epithelium of the developing lung and are restricted to large airways (not shown). In contrast, individual cells within the epithelium of the main bronchus and of bronchioles show abundant *MUC2* mRNA in adult lung. Hence it is unlikely that *MUC2* mucin plays a key role in early development of CF lung disease, though this mucin may contribute to large-airway pathology later in life.

Digestive system

Perhaps suprisingly, given the known importance of *MUC1* in the pancreatic duct epithelium post-natally we were unable to detect *MUC1* transcripts in the pancreas until term. These results were consistent for a number of pancreases of various ages, including some in which *CFTR* expression had been detected in adjacent tissue sections. This is in contrast with data from northern analysis (Batra et al., 1992), which suggested that *MUC1* was transcribed in 18- to 24-week pancreas. An explanation for this discrepancy remains to be found. Nonetheless, it is clear that cultured cells from mid-trimester foetal pancreatic ducts do express *MUC1* (Harris and Coleman, 1988) and a that a number of pancreatic adenocarcinoma cells lines express high levels of *MUC1* (Lan et al., 1991). It is possible that the high levels of *MUC1* expression seen in the adenocarcinoma cell lines are due to their tumour origin rather than the fact they arise from pancreatic duct epithelial cells. Expression of *MUC1* is known to be upregulated in a number of tumour tissues and cell lines (Kovarik et al., 1993; Abe and Kufe, 1993). At no stage in development was *MUC2* expression detected in the foetal pancreas.

Within the developing intestine both *MUC1* and *MUC2* transcripts are seen, though their patterns of distribution are different. *MUC1* expression appears in the colon around 16 weeks of gestation and continues to be expressed in the crypt epithelial cells through term and post-natally. At no stage is *MUC1* expression seen in the small intestine. *MUC2* is expressed throughout the intestinal epithelium by 12 weeks of gestation. The pattern of expression of *MUC2* is quite different from that of *MUC1*. *MUC1* is expressed in all crypt epithelial cells and in decreased amounts in cells that have migrated along the villi. This cellular localization of *MUC1* expression in the colon is similar to that seen for *CFTR*. High levels of expression of *MUC2* are seen within individual epithelial cells, possibly goblet cells, with adjacent cells showing little expression of the gene. This gives rise to a distinctive pattern of focal localization of silver grains on in situ hybridization. Epithelial cells that express high levels of *MUC2* are seen throughout the crypt and villus epithelium, though they are seen more frequently within the intestinal crypts. This localization of *MUC1* and *MUC2* mRNA suggests that both genes may be expressed in the epithelial stem cells at the base of the crypt and as these cells migrate along the villus and differentiate, *MUC1* expression is turned off and *MUC2* expression is maintained at a high level solely in goblet cells.

CFTR expression is seen within epithelial cells of the crypts in both small intestine and colon by 18 weeks of gestation, which is noteworthy because intestinal obstruction is often an early feature of CF pathology. Both *MUC1* and *MUC2* might contribute to intestinal obstruction in CF; however, since *MUC1* is only present in the colon it would be predicted to have a smaller role than *MUC2*. In addition, it is likely that other mucins that have not yet been completely characterized at the cDNA level, but appear to be expressed in intestinal tissues, are also involved. For example, it is known from *MUC2* and *MUC3* localization studies using monoclonal antibodies, that both these genes are expressed in adult jejunum, ileum and colon, though with different cellular localizations (Ho et al., 1993).

Urinogenital system

MUC1 expression is evident in the epithelium of collecting ducts within the kidney by 12.5 weeks of gestation. This pattern and level of expression remains constant throughout development, to term. *CFTR* is not expressed in human fetal kidney though CFTR protein is detectable in adult kidney epithelium with antibodies to CFTR (Crawford et al., 1992). However, the kidney is not apparently involved in the pathology of CF. No *MUC2* expression was observed in the kidney.

We have previously shown that *CFTR* is expressed in epithelial cells of the mid-trimester vas deferens and epididymis (Coleman and Harris, 1991; Trezise et al., 1993). Neither *MUC1* nor *MUC2* shows substantial expression in developing male genital ducts or in mid-trimester testis. Since obstruction of male genital ducts appears to be a diagnostic feature of CF this may suggest that other mucins may be involved. Alternatively, the absence of vas deferens that is characteristic of most CF males may be caused by a failure to develop a normal duct system rather than by obstruction of the ducts.

In conclusion we have shown that early CF pathology may involve *MUC1* in the respiratory system and both *MUC1* and *MUC2* mucins in the intestine. However, it is likely that other mucins may be involved in pancreatic disease and may additionally contribute to intestinal disease and genital duct defects. We have defined the localization of expression of the *MUC1* and *MUC2* genes during human development and correlated them with expression of *CFTR*. The identification of cells that express both *CFTR* and a specific mucin gene provides a logical starting point for further molecular analysis of mucin expression and glycosylation in specific epithelial cell types in cystic fibrosis.

We are grateful to Drs M. Dunhill, S. Gould, J. Hull, M. Seller and P. Tam for their help, Dr C. Basbaum for the pHAM1 probe, Dr S. Tebbutt for sequence analysis and Professor Richard Moxon for continued support. A.E.O.T. is a Beit Memorial Research Fellow. This work was supported by the Department of Paediatrics, Oxford University, the Cystic Fibrosis Research Trust and the following grants from the National Institutes of Health, USA (DK44762 and DK 46589).

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(Received 23 September 1993 - Accepted 5 November 1993)