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Transmembrane and truncated (SEC) isoforms of MUC1 in the human endometrium and Fallopian tube

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Abstract

The cell surface mucin MUC1 is expressed by endometrial epithelial cells with increased abundance in the secretory phase of the menstrual cycle, when it is found both at the apical cell surface and in secretions. This suggests the presence of a maternal cell surface glycoprotein barrier to embryo implantation, arising from the anti-adhesive property of MUC1. In previous work, we demonstrated alternatively spliced MUC1 variant forms in tumour cells. The variant MUC1/SEC lacks the transmembrane and cytoplasmic sequences found in the full-length variant. We now show that MUC1/SEC mRNA is present in endometrial carcinoma cell lines, endometrial tissue and primary cultured endometrial epithelial cells. The protein can be detected using isoform-specific antibodies in uterine flushings, suggesting release from endometrium in vivo. However, on the basis of immunolocalisation studies, MUC1/SEC also remains associated with the apical epithelial surface both in tissue and in cultured cells. Transmembrane MUC1 and MUC1/SEC are both strikingly localised to the apical surface of tubal epithelium. Thus MUC1 may contribute to the anti-adhesive character of the tubal surface, inhibiting ectopic implantation. The mechanism by which this barrier is overcome in endometrium at implantation is the subject of ongoing investigation.

Background

MUC1 is a high Mr, heavily glycosylated polymorphic glycoprotein expressed in a variety of epithelia, including the glandular and luminal epithelium of the endometrium [1–3]. It is encoded by a gene comprising 7 exons that span approximately 4 to 7 kb of DNA, depending on the number of 60 bp tandem repeat units within the variable number of tandem repeats (VNTR) domain located in exon 2 [1,4,5].

During the menstrual cycle the endometrium undergoes cyclic phases of proliferation and differentiation. MUC1 expression is up-regulated in the secretory phase, and remains high throughout the period of receptivity during which embryo implantation occurs [6]. It is also elevated in breast carcinoma relative to normal tissue [7] as well as in the normal differentiated state of lactation [8].

Immunohistochemistry using monoclonal antibodies (McAbs) to MUC1 core protein has shown that in

proliferative phase endometrium, MUC1 is largely confined to the apical surface of epithelial cells. The most well characterised gene product is a type I integral membrane glycoprotein (MUC1/REP/TM) [1,4,9]. However, in the early secretory phase, increased intracellular MUC1 immunoreactivity reflects increased synthesis, the appearance of specific glycoforms [10] and progressive accumulation in glandular secretions. By the mid to late secretory phase, abundant secretion of MUC1 occurs and a soluble form can be detected in increasing amounts in uterine fluid. At the same time however, significant amounts of MUC1 remain associated with the apical epithelial cell surface [3].

Two possible mechanisms have been proposed to account for the production of soluble MUC1. It is known that a proteolytic cleavage occurs during post-translational processing of the cell-associated molecule within the endoplasmic reticulum [2]; the two resulting subunits remain non-covalently associated. The cleavage site has been mapped to a site upstream of the transmembrane domain [2,11,12]. The products are a large N-terminal subunit containing the VNTR and a smaller C-terminal subunit containing 58 residues of the extracellular domain, together with the transmembrane and cytoplasmic domains. Release of the N-terminal subunit is thought to occur due to dissociation of the complex or as a result of a second cleavage. Evidence consistent with the release/shedding of the N-terminal subunit has been obtained from experiments in which full length MUC1 cDNA expression constructs were transfected into mouse cells [13]. MUC1 was identified in conditioned medium by radioimmunoassay and immunoprecipitation using anti-VNTR antibodies. Polyclonal antiserum against the cytoplasmic tail failed to precipitate the soluble form, supporting the hypothesis that release from the cell surface occurs either as a result of proteolytic cleavage in the membrane-proximal domain or simple shedding of the N-terminal subunit. Similar observations have been made in human epithelial cell lines [14].

An alternative explanation for secreted MUC1 molecules arose through the identification of a second MUC1 cDNA species, MUC1/SEC [9]. This shares much of the MUC1/REP/TM ectodomain, but 447 nucleotides distal to the VNTR domain, MUC1/SEC becomes co-linear with genomic DNA because intronic sequences between exon 2 and exon 3 are not spliced out. The first 33 nucleotides of the intron provide a short open reading frame with the capacity to encode a unique sequence of 11 amino acids. This transcript contains a long 3' UTR. The predicted translation product is a truncated form of MUC1 (MUC1/SEC) that lacks the transmembrane and cytoplasmic domains as well as the proteolytic cleavage site, and has the potential to be secreted directly from the cell. Other splice variants of the transmembrane form of MUC1 have also been identified [15,16].

Most of the evidence for alternative splicing of MUC1 has been obtained through work on breast carcinoma cell lines and primary breast cancer tissue. It is interesting to speculate whether the tissue type and state of differentiation may affect the pattern of splicing. Indeed, loss of MUC1/SEC expression has been associated with ovarian malignancy [16]. In the current study, an analysis of MUC1 alternative splicing in normal endometrial tissue and endometrial carcinoma cell lines was performed using a reverse transcription polymerase chain reaction (RT-PCR) strategy. Antibody reagents were used to verify translation from alternatively spliced transcripts in endometrial cell lines and tissue. Tubal tissue was also examined as it contains a closely related epithelium with discrete functions. The association of MUC1/SEC with the cell surface in all tissues examined indicates that it contributes to the glycocalyx of upper reproductive tract epithelia.

Materials and Methods

Cell lines

Endometrial carcinoma cell lines HEC1A (American type culture collection, HTB-112) and HEC1B (HTB-113) were obtained from Dr. Andrew Sharkey, University of Cambridge. HEC1A cells were maintained in McCoy's 5a medium (GibcoBRL) containing 10% foetal bovine serum (FBS; Advanced Protein Products). HEC1B cells were maintained in Eagle's MEM supplemented with Earle's balanced salt solution, 10% FBS and 1 mM sodium pyruvate (GibcoBRL). The Ishikawa cell line was obtained from Dr. John White, IRDB, Hammersmith Hospital, London, and maintained in DMEM containing 20 mM HEPES (GibcoBRL) and 10% FBS. The human breast carcinoma cell line T47D was obtained from Dr. Michael Daws, University of Newcastle Upon Tyne. Cells were maintained in DMEM containing 10% FBS and 1 μg/ml insulin. In addition, all media were supplemented with glutamine (2 mM; ICN) and antibiotics: streptomycin (100 μg/ml; Evans Medical Ltd) and gentamycin (5 μg/ml; David Bull Laboratories).

Uterine flushings

Uterine flushing was performed as an out-patient procedure at the gynaecology clinic of the Jessop Hospital for Women, Sheffield under local ethical committee approval. The procedure and collection criteria were as previously described [3,17].

Endometrial primary culture

Endometrial epithelial cells (EEC) were grown from isolated endometrial glands purified as previously described [18,19]. Cells were cultured and grown to confluence in steroid-depleted medium: 75% DMEM (Sigma, St. Louis,

MO) and 25% MCDB-105 (Sigma) containing antibiotics, supplemented with 10% charcoal-Dextran treated FBS (Hyclone, Logan, UT) and 5 μg/ml insulin (Sigma) as described [18].

Endometrial tissue and cell monolayer RNA extraction

Endometrial biopsy material obtained at dilatation and curettage under local ethical committee approval was collected directly into 2 ml guanidinium thiocyanate solution (4 M guanidinium thiocyanate, 0.5% N-lauroylsarcosine, 0.1 M 2-mercaptoethanol, 0.1% Antifoam A and 25 mM sodium citrate pH 7.0) and homogenised with a Polytron X-1020 (Brinkmann Instruments). Denatured, homogenised samples were stored at -80°C prior to RNA extraction. Cells were grown to confluence in 75 cm^2 culture flasks (Falcon). Medium was removed and cells washed 3 times in ice-cold PBS (pH 7.4). Guanidinium thiocyanate solution (5 ml) was added to the flasks, which were agitated for approximately 2 min. This solution was then transferred into a 50 ml universal (Falcon) and stored as above. Total RNA was isolated using the acid guanidinium thiocyanate-phenol-chloroform method [20].

Purification of mRNA from total RNA

Poly (A^+) RNA was purified from total RNA using pre-prepared oligo d(T)-cellulose spin columns (mRNA Purification Kit, Pharmacia Biotech) as directed by the manufacturer. Briefly, total RNA sample was dissolved in elution buffer (10 mM Tris-HCl (pH 7.4), 1 mM EDTA) and heated to 65°C for 5 min. After rapid cooling on ice, a 200 μl aliquot of sample buffer (10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 3 M NaCl) was added to the RNA sample, which was then applied to the oligo d(T)-cellulose column, pre-equilibrated with high salt buffer (10 mM Tris-HCl; pH 7.4, 1 mM EDTA, 0.5 M NaCl). After the sample had entered the cellulose matrix, the column was spun at 350 g for 2 min. Two washes with high salt buffer followed by two washes in low salt buffer (10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.1 M NaCl) were performed before elution of bound poly $(A)^+$ RNA was effected by the application of four 250 μl aliquots of elution buffer, prewarmed to 65°C. For the analysis of alternative splicing it was important to eliminate genomic DNA. To this end, two rounds of column purification of poly$(A)^+$ RNA were performed.

First strand cDNA synthesis

Reactions were performed in a volume of 20 μl which contained 10 μg of total RNA, diluted to a total volume of 8 μl in DEPC-treated water or 8 μl of oligo d(T)-cellulose column eluate containing poly (A^+). Samples were heated to 65°C for 10 min and rapidly cooled on ice for 10 min. The following reagents were then added sequentially: 4 μl 5 × RNA buffer (GibcoBRL; 250 mM Tris/HCl pH 8.3 at room temperature, 375 mM KCl, 15 mM $MgCl_2$), 2 μl 0.1 M DTT (GibcoBRL), 2 μl 4 mM (0.05 μg/μl) oligo d(T) primer (16–28 nucleotides; Pharmacia Biotech), 2 μl 10 mM of each dNTP (40 mM total; a 1/10 dilution 0f 100 mM stock dNTP's, 8 μl of each dNTP in 48 μl of DEPC-treated water; Pharmacia Biotech), 1 μl (30 units) RNA Guard (Pharmacia Biotech) and finally 1 μl (200 units) Moloney Murine Leukaemia Virus reverse transcriptase (RT; GibcoBRL). The reaction was incubated at 37°C for 1 hour and then terminated by incubation at 75°C for 10 min in order to denature RT. After centrifugation for 1 min at 13,000 rpm in an MSE microfuge the reaction mixture was stored at -80°C.

Primers to identify a MUC1/REP/TM transcript

The complete mRNA sequence of human polymorphic epithelial mucin (MUC1) (GenBank J05581) was used to design a primer pair for the identification of MUC1 transcripts encoding a transmembrane domain. The upstream primer 5'-AGC ACC GAC TAC TAC CAA G-3' (primer 1 in Fig. 1B) hybridises at position 916 of J05581. The downstream primer 5'-CTG AGA AGT GTC CGA GAA A-3' (primer 2 in Fig 1B) hybridises at position 1649. This primer pair annealed at 55°C to generate a product of 752 bp.

Primers to identify a MUC1/SEC transcript

An upstream primer was designed using the mRNA sequence of human secreted epithelial tumour mucin antigen (MUC1; GenBank accession number X52228). Primer 4 in Fig. 1A, 5'-TGG AGA CAC AGT TCA ATC AGT AT-3' hybridises at position 1436 in exon 4. The downstream primer (3 in Fig. 1A), 5'-TGC TCA CTT CAC GCC ACT T-3' hybridises at position 3209 of Genbank sequence M35093 (MUC1/SEC). This primer pair annealed at 57°C to generate a product of 686 bp. Genomic DNA was not amplified under the conditions used.

Primers required for specific sequence amplification

Primers were designed to enable amplification of intron VI sequence from MUC1. The upstream primer 5'-AAG TGG CGT GAA GTG AGC A-3' hybridises at position 3302 of Genbank sequence M61170, while the downstream primer 5'-GCC TCT GGG TGC AAA AAA-3' hybridises at position 3521. This primer pair anneals at 56°C to generate a product of 237 bp.

Polymerase Chain Reaction (PCR)

PCR's were carried out in 20 μl reaction volumes. Each reaction contained: 2 μl each of the forward and reverse primers (both at 5 μM), 2 μl 10 × PCR buffer (0.85 mg/ml BSA, 670 mM Tris/HCl pH 9.0 at 25°C, 166 mM ammonium sulphate and 1% Triton-X100), 1.5 μl 25 mM $MgCl_2$, 3.0 μl 2 mM dNTP mixture (each at 2 mM), 0.1 μl of 10 units/μl TAQ DNA polymerase (0.5 units; Promega)

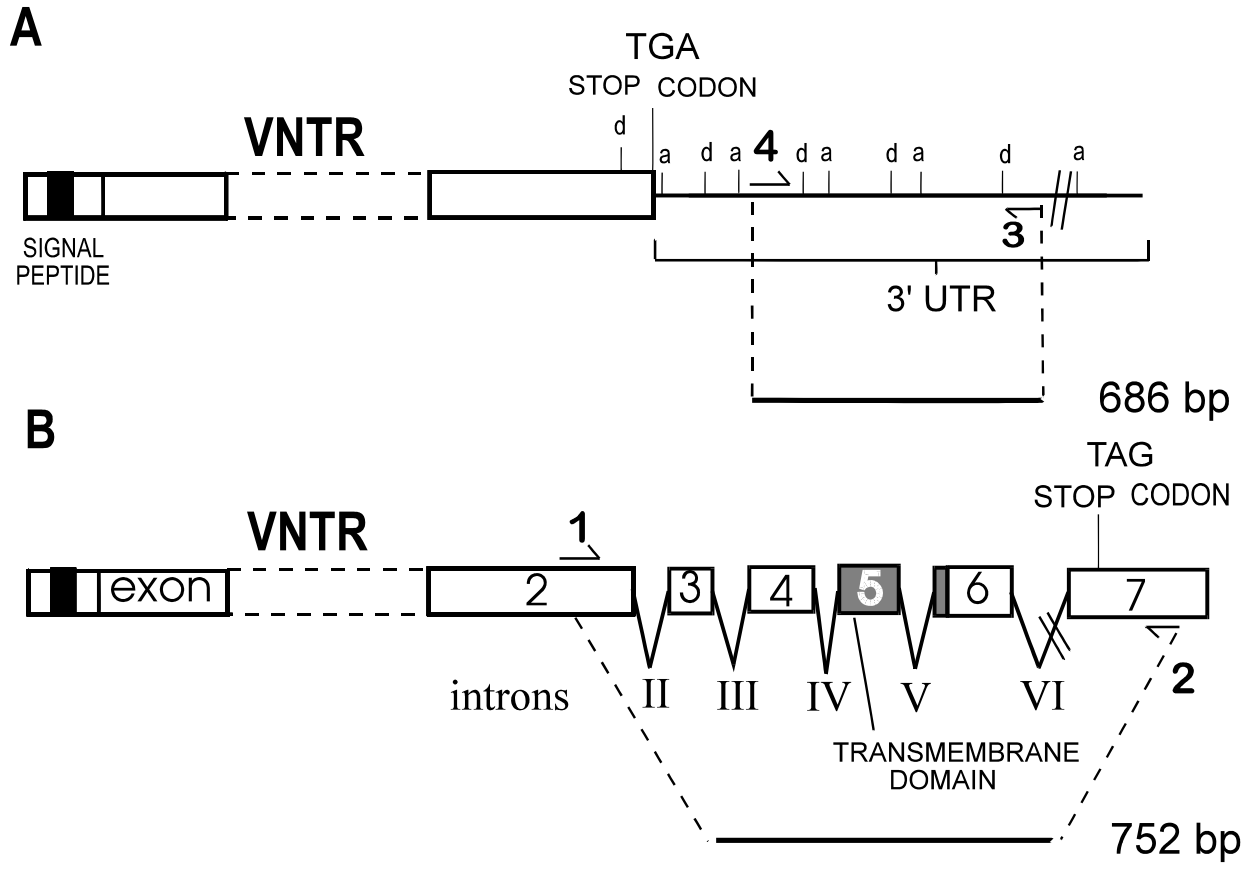

Figure 1
Exon-intron structure 3' of the VNTR that generates the secretory (A) and cell-associated (B) isoforms of MUC1. The letters 'd' and 'a' refer to splice donor and acceptor sites. Bars represent translated sequence and lines represent non-coding mRNA (A) and removed introns (B). Sites of primer annealing and the sizes of predicted amplification products are indicated.

and 7.4 μl sterile ultra-pure water to increase the reaction volume to 18 μl. The reaction mixture was overlaid with liquid paraffin to avoid evaporation of components during thermal cycling. Finally, 2 μl of RT reaction mixture containing cDNA (~1 μg) was added and contents of the reaction vessel centrifuged before thermal cycling. Amplification reactions were performed in a Microprocessor Controlled Incubation System (Appligene). Negative controls in which cDNA was replaced by sterile ultra-pure water were included in every series of reactions to ensure that reagents were free from contaminating DNA. Thermal cycling conditions were 95°C for 2 min, 35 cycles of 95°C for 1 min, primer annealing for 1 min at the described temperatures, 72°C for 1 min and a final 72°C incubation for 5 min.

PCR reactions were analysed by electrophoresis through gels of 1% (w/v) agarose (Molecular biology grade, Sigma) in 1 × Tris/acetate EDTA (TAE; 40 mM Tris, 21 mM glacial acetic acid, 5 mM sodium EDTA) containing 0.5 μg/ml ethidium bromide. DNA was visualised by ethidium bromide fluorescence on exposure to ultra-violet light. Inclusion of a DNA 100 bp ladder (Pharmacia Biotech) enabled sizing of PCR products.

Products were purified for restriction analysis and for use as templates in subsequent PCR reactions. When a single product was generated by PCR it was purified by chloroform extraction and ethanol precipitation. However, when multiple products were generated electrophoretic separation was performed and the product of interest

excised from the gel and purified from the agarose slice using the Qiaex agarose gel extraction kit (Qiagen).

Restriction digestion and sequencing

Nco I (10 units at 5000 units/ml; Pharmacia Biotech) was used in 1 × One-Phor-All Plus Buffer (10 mM Tris-acetate pH 7.5, 10 mM magnesium acetate and 50 mM potassium acetate). Sequencing of PCR Products was performed using a Sequenase Kit (United States Biochemical) as directed by the manufacturer.

Dual determinant Enzyme Linked Immunosorbant Assay (ELISA)

ELISAs were performed as previously described [3]. Briefly, HPLC-purified BC2 (IgG) was employed as a capture antibody and was coated on 96 well plates (Dynatech Immulon) at a concentration of 4 μg/ml in PBS (pH 7.4), 100 μl/well for 4 h at room temperature. BC2 recognises the repeating epitope PDTRP within the MUC1 VNTR domain. Non-specific sites were blocked with either PBS containing 5% sucrose and 0.5% bovine serum albumin (v/v), or 5% milk powder, 100 μl/well for 2 h at room temperature. Wells were washed in 100 μl of PBS (pH 7.4) containing 0.05% (v/v) Tween 20 (PBS-T) and 50 μl/well of sample applied for an overnight incubation at 4°C. After removal of sample, wells were washed in three 10 min changes of PBS-T. In the conventional ELISA a second anti-repeat McAb BC3 (IgM) was used as detector at 1/1000 dilution of ascites in PBS. Alternatively, McAb 7H10/5 (IgM) raised against MUC1/SEC specific peptide VSIGLSFPMLP [21], was used as detector at 1/1000 dilution of ascites in PBS. Incubation with 50 μl/well detector antibody was performed for 2 h at room temperature and followed by 3 × 10 min washes in PBS-T. Finally, a peroxidase-conjugated rabbit anti-mouse IgM antibody (μ chain specific; Nordic) was used at 1:500 in PBS, 50 μl/well. After two 5 min washes in PBS-T and 5 min in citrate-phosphate buffer (0.1 M citric acid, 0.1 M disodium hydrogen phosphate pH 5.0) 100 μl of substrate reagent 2 mM 2,2'azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Boehringer Mannheim) containing 0.02% hydrogen peroxide in citrate phosphate buffer pH 5.0 was applied to the wells. Reaction product was measured by absorbance at 405 nm in an automated plate reader (Bio-Tek Instruments) after 20 min colour development. Negative controls were employed in which capture and detector antibodies were individually omitted from the assay, together with the replacement of sample by PBS.

Immunofluorescence

Tissues were obtained at specific days of the luteal phase monitored from the LH peak. Endometrial and tubal tissue was snap-frozen in liquid nitrogen and 6 μm cryosections produced and stored at -80°. Sections were fixed in cold methanol, 5 min, for anti-repeat MUC1 antibodies or the unique epitope recognised by antibody 232A/1, which is in the membrane-proximal domain of MUC1/REP/TM [11]. The two monoclonal antibodies to MUC1/SEC did not react with aldehyde-fixed (including routinely processed) or methanol-fixed tissue; for these reagents cold acetone fixative was applied for 5 min, then sections treated with PBS/4% BSA to block non-specific protein binding. Carcinoma cell lines were cultured on multichamber glass slides, while EEC were cultured in tissue culture plastic wells. In each case fixation was with methanol. Staining procedures as previously described [22]. First and second antibodies were diluted in PBS/BSA. Controls carried out with isotype-matched first antibodies were negative in all cases.

Results

Identification of transcripts predicted to encode both cell-associated and secretory isoforms of MUC1

Primers were designed to identify splice forms of MUC1 (Fig. 1). Primers 1 and 2 complementary to sequences in exons 2 and 7 respectively were used to confirm the presence of the classical product MUC1/REP/TM from which introns II to VI had been spliced out. (Fig. 1B). A single product of the predicted size (752 bp) was produced from cDNA prepared from normal endometrial tissue throughout the menstrual cycle and also in cDNA from Ishikawa, HEC1A and HEC1B endometrial carcinoma cells (not shown). Restriction analysis was performed using Nco I, which was predicted to cut at a single site at nucleotide 459. The 752 bp product yielded two fragments in the predicted size range of 274 bp and 459 bp.

A new 3' primer (Fig. 1A) was designed to anneal within the 3' untranslated region (UTR) of the transcript predicted to encode a truncated, secretory isoform of MUC1 (MUC1/SEC). The priming site lies within intron VI between exons 6 and 7 in the genomic sequence, and is therefore unique to this transcript. Coupled with a 5' primer that anneals at a site in exon 4 (Fig. 1A), this primer was predicted to generate a PCR product of 686 bp. The expected product was obtained using cDNA from endometrium (Fig. 2A) or endometrial carcinoma cell lines (Fig. 2B). Additional products were also generated, the most abundant of which was 463 bp. Slight differences in the relative abundance of products were observed, but no obvious correlation was noted with the cycle stage of the tissue from which the cDNA was derived.

The transcript predicted to encode MUC1/SEC is co-linear with the gene. To discount the possibility of genomic DNA contamination of total RNA, poly (A^+) RNA was purified by two successive oligo d(T) column separations and used in RT-PCR reactions. Identical products were generated using cDNA derived from poly (A^+) and total RNA, confirming that these products were derived from

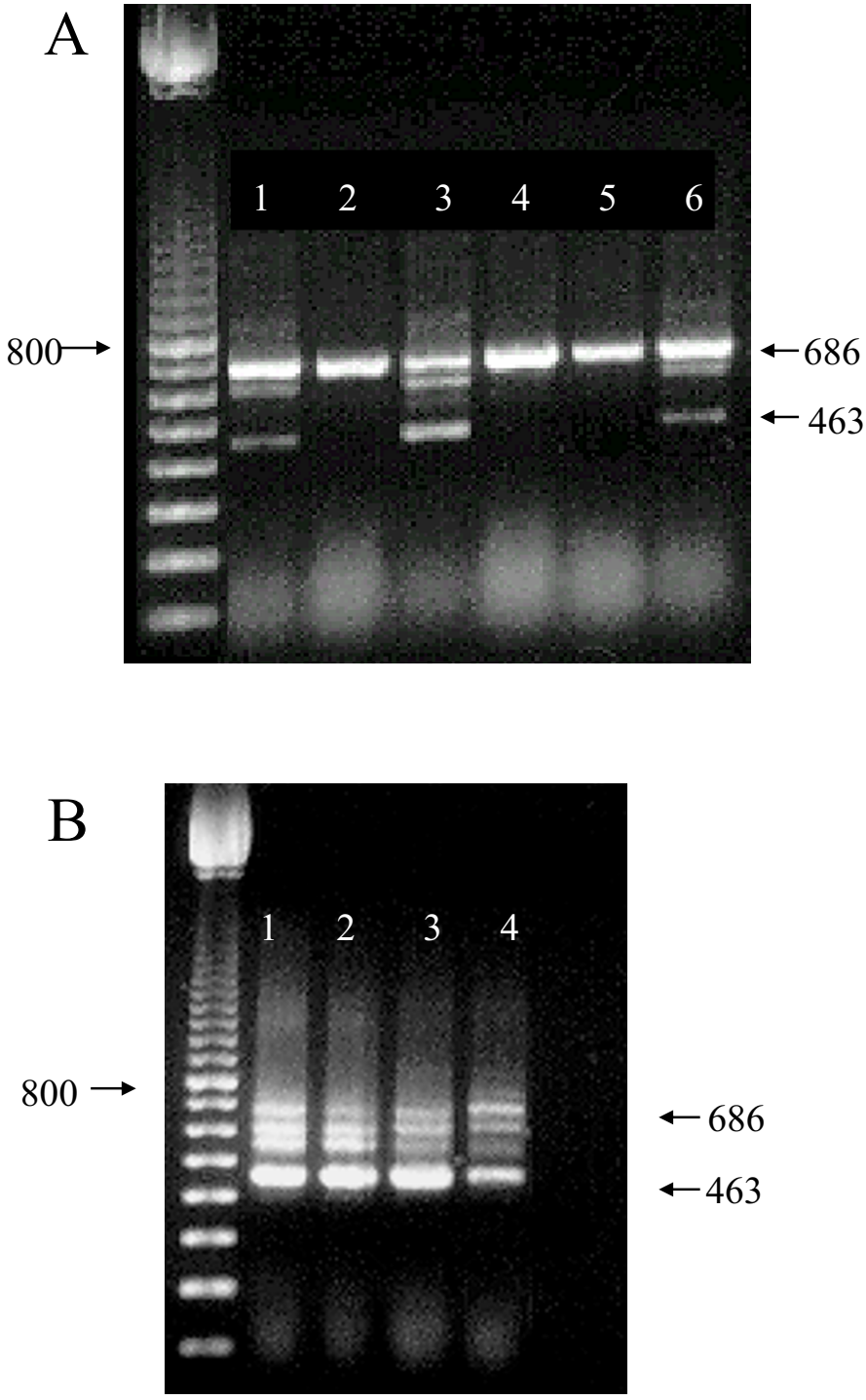

Figure 2
Results of RT-PCR using primers 4 and 3 (see Fig. 1A) with: (A) 6 independent samples of secretory phase endometrial tissue cDNA. Lanes 1 and 2 are early, 3 and 4 mid, and 5 and 6 late secretory phase samples. Different patterns of product relative abundance were not found in particular phases. (B) RT-PCR using endometrial carcinoma cell lines HEC1A (1), HEC1B (2), AN3-CA (3) and the breast carcinoma cell line T47D (4). Controls in which cDNA was omitted were always negative (B, lane 5). The 686 bp product is predicted to encode a secretory isoform of MUC1.

mRNA and not from genomic DNA (not shown). The MUC1/SEC mRNA is predicted to be larger than that encoding normal MUC1. However we were unable to detect abundant larger products on Northern blots using probes either to the VNTR domain (Hey *et al.*, 1994) or to SEC-specific sequences (not shown). This suggests that the SEC mRNA species is expressed at a lower level than transmembrane MUC1 mRNA.

The smaller product of 463 bp was re-amplified, purified and sequenced in both directions. The sequence was in accord with the Genbank X52228 intron VI sequence except t* for g and g* for a substitutions in the sequence aag ggt* ccc tca ag*a ggg. This confirmed that it arose from a transcript that lacks introns II to V, but contains intron VI sequence.

If translated, the transmembrane product encoded by this novel spliced mRNA would harbour an extended cytoplasmic tail of 183 amino acids, the first 50 of which are encoded by exon 6 and are identical to the conventional C-tail. An additional novel sequence of 133 amino acids could be encoded by the open reading frame of intron VI. A polyclonal antiserum raised against a 17 amino acid synthetic peptide representing the C-terminal residues of the novel C-tail reacted strongly with the immunising peptide but a native MUC1 protein could not be detected either in endometrial carcinoma cell lines or tissue sections of endometrium. It could be that the polyclonal antibody preparation directed against the synthetic peptide does not recognise this epitope when presented in the context of the putative native MUC1 protein. Alternatively, the lack of immunoreactivity may indicate a low level of transcription or translation. Further investigations will be required to clarify whether this novel MUC1 protein is indeed present.

Identification of MUC1/SEC protein in a double determinant ELISA

Expression of the MUC1/SEC protein was examined using two McAbs raised against the unique 11 mer peptide (VSIGLSFPMLP) present at its C-terminus and corresponding to the sequence encoded by intron II [21]. McAb 7H10/5 was used in combination with the anti-tandem repeat McAb BC2 in a double determinant ELISA to determine whether the "soluble" form of MUC1 previously detected in uterine flushings [3] is MUC1/SEC. Analysis of 8 secretory phase uterine flushings using the conventional MUC1 ELISA (anti-VNTR capture and detector) confirmed the presence of variable amounts of MUC1 in all samples assayed (Fig. 3). Substitution of BC3 with the anti-MUC1/SEC-specific McAb 7H10/5 revealed that a proportion of the MUC1 signal is generated by MUC1/SEC protein. Quantitative comparisons are not possible because anti-VNTR detector antibodies can potentially bind to multiple sites, whereas the anti-MUC1/SEC binds at a single epitope. However, differences in the signal produced by the two detector antibodies in different specimens suggest that a proportion of the soluble MUC1 may be derived from an isoform not detected by McAb 7H10/5.

Immunofluorescent detection of MUC1/SEC in epithelial cells

Initial experiments were carried out to ascertain optimal conditions for the detection of MUC1/SEC in tissue sections. Negative results were obtained after fixation with aldehyde or alcohol, but strong immunofluorescence was observed after fixation in acetone. This confirmed that MUC1/SEC is present at the apical surface of both glandular and luminal epithelial cells in endometrium but appears more abundant in tubal tissue where the distribution is again exclusively apical (Fig. 4). Similar results were obtained with the two different SEC-specific monoclonal antibodies. MUC1/SEC co-distributes in tubal tissue with transmembrane MUC1/REP/TM detected using an antibody to the cytoplasmic tail (not shown). Staining is present in the fimbrial (Fig. 4A,4B), ampullary (Fig. 4C,4D) and isthmic (not shown) region of the tube and appears to be associated with ciliated cells in all locations. Using the same specific fixation protocol, MUC1/SEC is also associated with the surface of both primary cultured endometrial epithelial cells (Fig. 5A) and HEC1B cells (Fig. 5A,5B,5C,5D) where a heterogeneous distribution is apparent. A greater proportion of cells are immunopositive when assayed with antibody BC3 to tandem repeat sequences present in both MUC1/SEC and MUC1/TM/REP, than with antibody to a unique MUC1/SEC determinant (Fig. 5b,5c), indicating that some cells may contain transmembrane MUC1 and not MUC1/SEC. Antibody 232A/1, which recognises a unique (non-repeat) determinant on MUC1/TM/REP, binds a greater proportion of cells than antibody 7H10/5 to MUC1/SEC (Fig. 5b,5d), suggesting that the transmembrane form may predominate in culture.

Discussion

MUC1 expression by endometrial glandular epithelium is regulated during the menstrual cycle, with an increase in abundance of full length, VNTR-containing mRNA and protein (MUC/TM/REP) during the post-ovulatory, secretory phase [6]. MUC1 immunoreactivity is associated with the apical epithelial cell surface of glandular and luminal epithelial cells in both proliferative and secretory phases [3,6]. In the mid-secretory phase, levels of soluble MUC1 begin to increase in uterine fluid [3]. Here we have devised a reverse transcriptase PCR strategy that confirms the presence of the transmembrane isoform MUC1/TM/REP and indicates the existence of an alternative splicing mechanism that accounts for the production of an isoform MUC1/SEC that lacks the transmembrane and

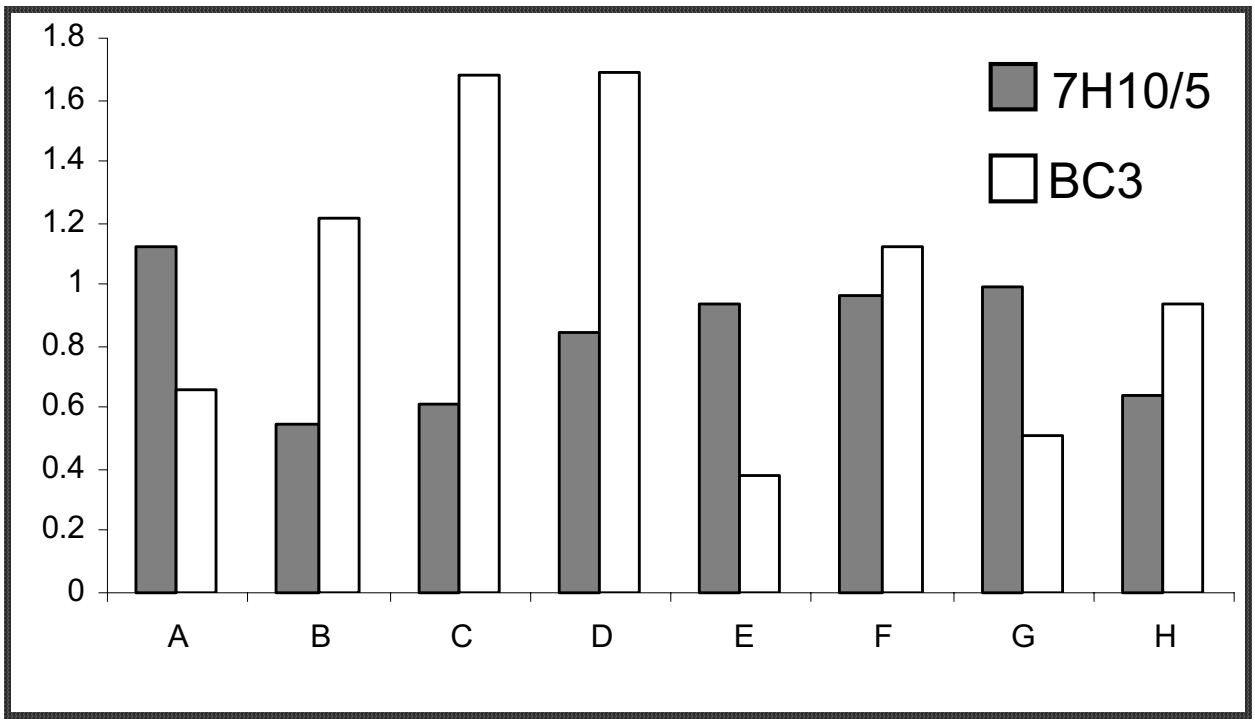

Figure 3
Analysis of uterine flushings using a double determinant ELISA. Anti-VNTR McAb BC2 was used as capture antibody with either anti-VNTR McAb BC3 (white bars) or anti-MUC1/SEC McAb 7H10/5 (grey bars) as detector antibodies. Use of 7H10/5 results in the identification VNTR-containing MUC1/SEC species, thus confirming expression of the alternatively spliced secretory isoform in endometrium. The vertical axis represents absorbance at 405 nm. Individual controls in which capture, uterine flushings and detector antibodies were omitted from the assay were performed for each antibody. For BC3 the mean control absorbance values obtained was 0.172 (sd = 0.0408) and for 7H10/5 was 0.316 (sd = 0.145).

intracellular domains. Further, we have investigated these different isoforms of MUC1 in normal endometrium and Fallopian tube, and in endometrial carcinoma cell lines.

A transcript predicted to encode MUC1/SEC was identified in endometrial cDNA from across the menstrual cycle and also in a variety of cell lines. The results of RT-PCR were confirmed by the use of two McAbs that recognise an 11 amino acid sequence unique to MUC1/SEC [21]. A double determinant ELISA enabled the detection of TR-containing MUC1/SEC molecules in secretory phase uterine flushings. MUC1/SEC protein has also been detected immunochemically in sera of breast cancer patients and in medium conditioned by T47D breast cancer cells [18] suggesting that it might account for the secreted MUC1 observed in tissue sections and uterine fluid [6]. Immunofluorescence also reveals MUC1/SEC in gland secretions. However, variation in the signal generated in uterine flushings by MUC1/SEC relative to that from the conventional anti-TR ELISA suggests that secreted MUC1 may not comprise exclusively MUC1/SEC. Proteolytic cleavage of surface-associated MUC1 has been postulated as an alternative mechanism of release [2].

In immunofluorescence, MUC1/SEC is readily detectable in association with the apical surface of endometrial epithelial cells. It is also displayed at the surface of tubal epithelial cells in vivo, and in vitro on primary endometrial epithelial cells and HEC1B cells. MUC1/SEC is known to bind with high affinity to another isoform, MUC1/Y, which arises from the same gene by another splicing pathway [23]. We have evidence for the presence of the MUC1/Y mRNA in endometrium [3]. The apical surface expression of MUC1/SEC may be accounted for by the presence of MUC1/Y in the plasma membrane, though further experiments will be required to verify this hypothesis.

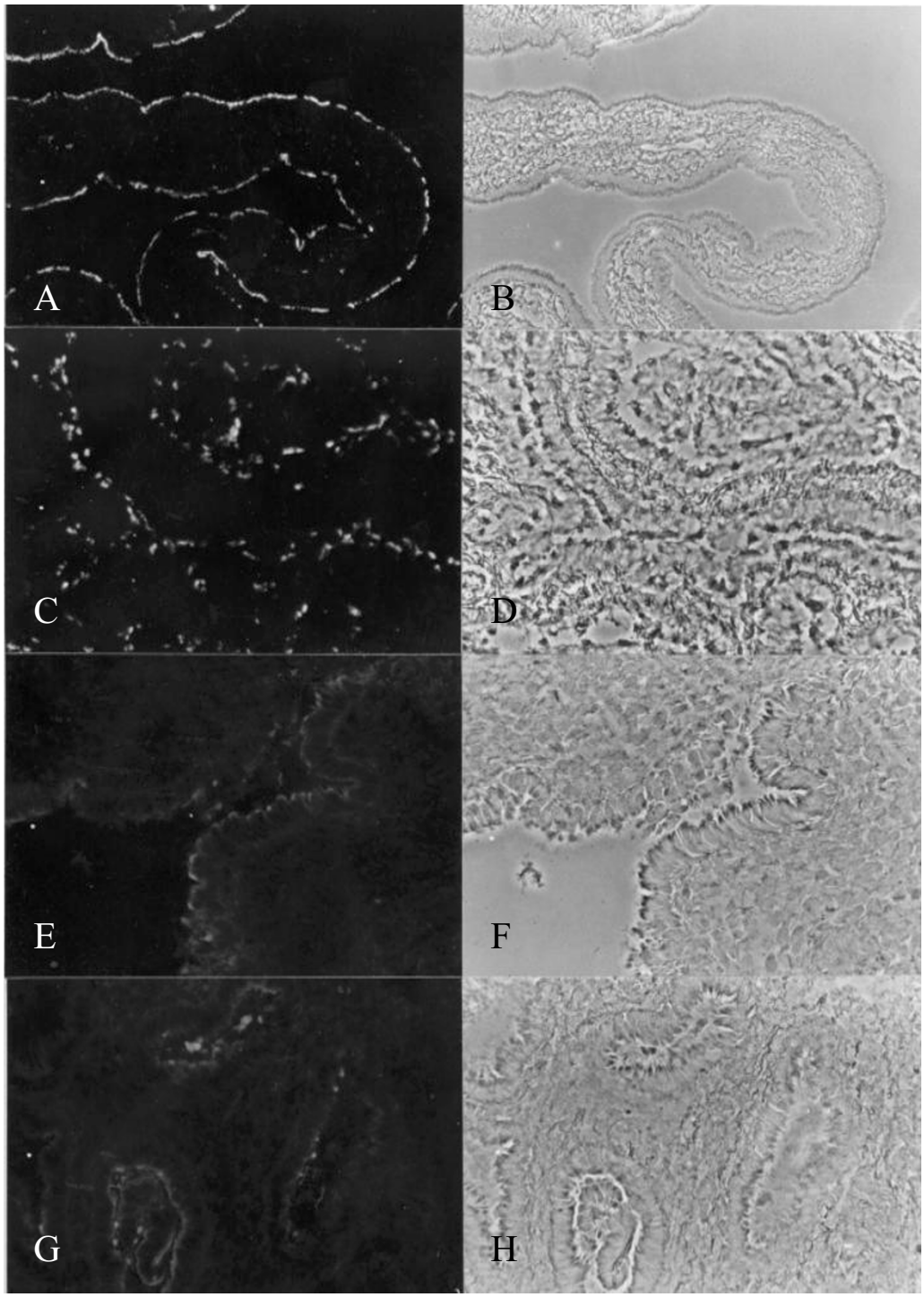

Figure 4
Immunofluorescence (A,C,E,G) and matching phase contrast (B,D,F,H) micrographs of (A,B) tubal (fimbrial) tissue (luteal phase day 3), (C,D) tubal (ampullary) tissue (luteal phase day 8) and (E-H) mid secretory endometrium (luteal phase day 8) stained with McAb 7H10/5. The antibody recognises the unique C-terminal peptide of MUC1/SEC. The interrupted pattern of staining in the tubal epithelium (A,C) reflects an association with ciliated cells. Staining is apparent at the apical surface of both luminal (E) and glandular (G) endometrial epithelium, and is also associated with secretory material.

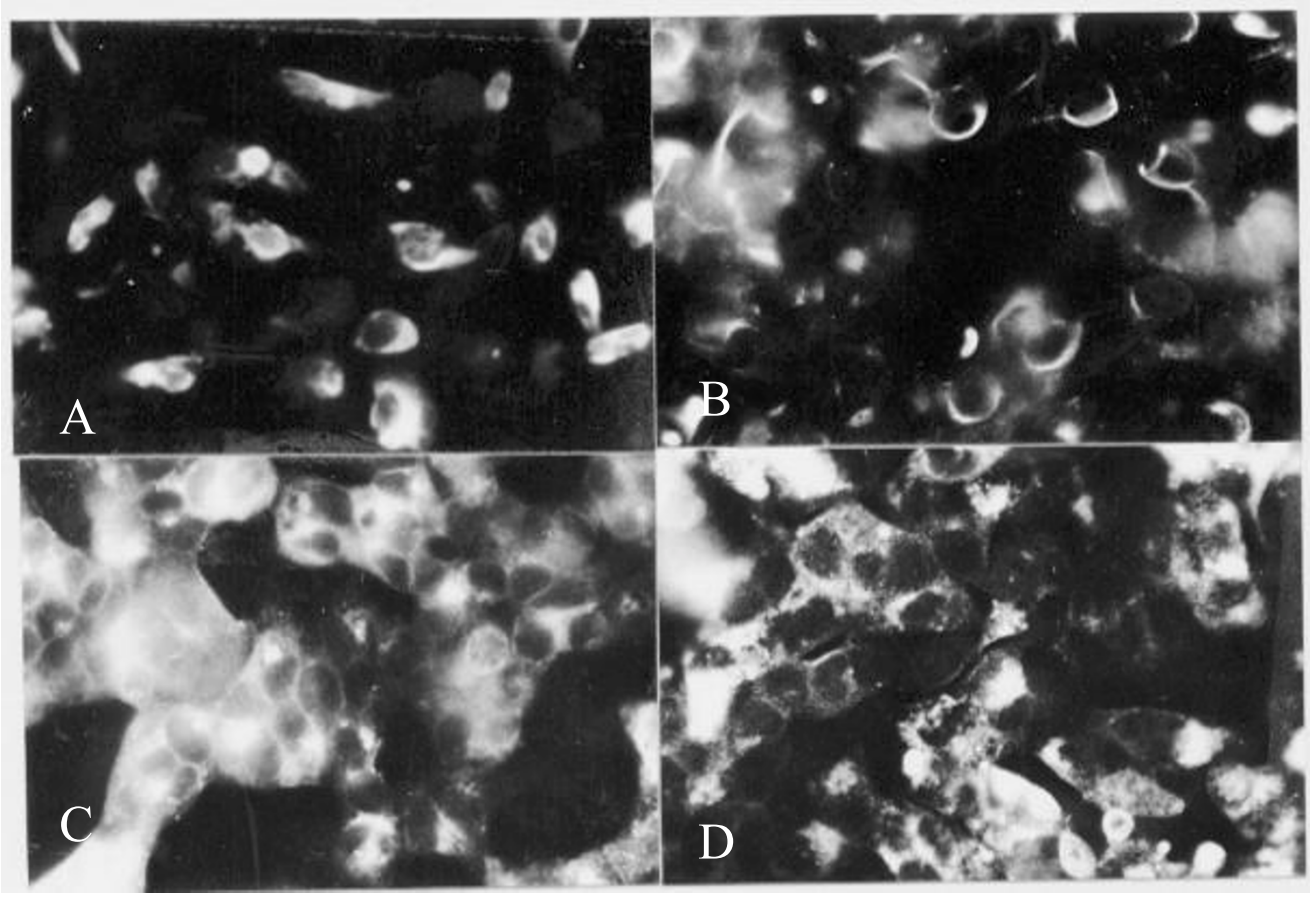

Figure 5
Immunofluorescence of primary cultured endometrial epithelial cells (A) and HEC1B cells (B-D) stained with antibodies against MUC1/SEC (A,B; Mab 7H10/5)), MUC1 tandem repeat domain (C; Mab BC3)) and a unique determinant in the ectodomain (D; Mab 232A/1). All fields contain confluent cells and the staining in each case is heterogeneous. The cell surface association of MUC1/SEC is particularly obvious in B.

The apical surface expression of different MUC1 gene products in the upper reproductive tract suggests the possibility of a role in relation to embryo transport and implantation. High levels of MUC1 at the cell surface reduce cell-cell and cell-matrix interactions, probably through steric hindrance [24,25]. This could create a barrier to embryo implantation, and thus help explain the non-receptivity of normal tubal tissue as well as the inability of donor embryos when transferred to the uterine cavity at inappropriate cycle times [26,27]. Further evidence for the role of MUC1 as a barrier component is provided by the observation that mouse embryos attach more readily to epithelial cells from Muc1-null mice [28]. In a primate model where ectopic implantation is unknown, we observed MUC1/SEC in tubal epithelium but not in the uterus, indicating the possibility of a greater barrier to attachment at the tubal surface [29]. Rabbit endometrium, like human, has a high level of MUC1 expression at the surface in early pregnancy, but this is down-regulated in the implantation site, probably as a result of paracrine signals from the embryo [30]. Local removal of MUC1 is predicted to allow receptor-mediated cell-cell interaction to proceed between the trophectoderm and the maternal epithelium, attaching the embryo to the apical epithelial cell surface in the first stage of implantation. This may occur by means of an apical surface reorganisation with the appearance of pinopods (also known as uterodomes [31]) that have been reported to lack MUC1 immunoreactivity [32]. Observations from an in vitro model of human implantation also suggest that a signal from the embryo may stimulate local loss of MUC1 at the site of implantation [33]. Results presented in the current study suggest a hy-

pothesis that removal of cell surface-associated MUC1/SEC as well as transmembrane MUC1 is required in order to permit implantation. They also suggest that receptivity might be defined as the ability of a steroidally-sensitised epithelium to respond to an embryonic stimulus; this ability is predicted to differ between tubal and endometrial locations.

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