The Mouse One P-Domain (*pS2*) and Two P-Domain (*mSP*) Genes Exhibit Distinct Patterns of Expression

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Abstract. We have previously shown that the human pS2 gene, which codes for a secreted peptide of 60 amino acids, is expressed in a number of human carcinomas, including carcinomas of the breast, the pancreas, and the large bowel. Strong *pS2* gene expression was also observed in the normal gastric mucosa and in the regenerative tissues surrounding ulcerous lesions of the gastrointestinal tract. A number of pS2 similar peptides, designated as P-domain peptides, have been described, notably the porcine (PSP), murine (mSP), and human (hSP) spasmolytic polypeptides, which correspond to duplicated pS2 proteins. We have now cloned a mouse homolog of the human *pS2* cDNA to dispose of an animal model to study the pS2 protein function, which remains unknown at the present time. We show that the mouse putative pS2 protein sequence and the physiological pattern of expression of the mouse *pS2* gene are well conserved. The mouse *pS2* gene is highly expressed in the stomach mucosa cells, whereas no *pS2* gene expression could be detected in the mouse mammary gland, even during postnatal development processes dependent on growth factors or hormones. Using in situ hybridization, we show that although coexpressed in the fundus, the antrum and the antrum–pyloric regions of the stomach, the mouse *pS2* and *mSP* genes exhibit distinct and complementary cellular patterns of expression.

THE human *pS2* gene codes for a small preprotein of 84 amino acids, containing at its amino terminus a signal peptide characteristic of secreted proteins (Masiakowski et al. 1982; Jakowlev et al., 1984). The secreted mature protein is 60 amino acids long (Rio et al., 1988*a*). The pattern of expression of the human *pS2* gene, in normal and pathological tissues, is well documented (for review see Rio and Chambon, 1990). The *pS2* gene was first reported to be expressed in 50% of human breast carcinomas, and in some of their associated lymph node metastases (Rio et al., 1987), but its expression is not specific to estrogen receptor positive carcinomas of the mammary gland, as pS2 mRNA and protein were also observed in carcinomas of the pancreas (70–75%) (Welter et al., 1991; Henry et al., 1991*a*), stomach (50–55%) (Luqmani et al., 1989; Henry et al., 1991*a*), large bowel (58%) (Henry et al., 1991*a*), biliary tract (Seitz et al., 1991), and in a mucinous subtype of ovarian tumors (Guerin et al., 1990; Theisinger et al., 1991; Henry et al., 1991*a*). The *pS2* gene was also shown to be physiologicaly expressed in the normal gastric mucosa (Rio et al., 1988*b*), and in the regenerative tissues surrounding ulcerous lesions of the human gastrointestinal tract (Rio et al., 1991).

In all tissues expressing the pS2 protein, immunocytological staining was observed in the cytoplasm of epithelial cells, with a preferential perinuclear accumulation. The regulation of *pS2* gene expression is tissue specific. In in vitro culture of tumoral mammary epithelial cells, *pS2* gene expression is estrogen-dependent (Berry et al., 1989). In vivo, the pS2 protein is predominantly expressed in estrogen receptor-positive breast carcinomas (Rio et al., 1987; Henry et al., 1989; Wysocki et al., 1990), and is indicative of a favorable response to hormonotherapy (Henry et al., 1989, 1991*b*; Schwartz et al., 1991; Klijn et al., 1992). However, no estradiol receptors have been found in the gastrointestinal tract and, in this tissue, the expression of the *pS2* gene could be dependent on EGF (Wright et al., 1990*a*). In fact, the 5′ flanking region of the *pS2* gene contains a complex enhancer region responsive to oestrogen, EGF, the tumor promoter TPA, and the proto-oncoproteins c-Ha-ras and c-jun (Nunez et al., 1989).

Very little is known concerning the possible function of the

pS2 gene product. Sequence similarity has been reported with the porcine pancreatic spasmolytic polypeptide (PSP)[1] (Thim, 1988; Backer, 1988), which appears to correspond to a duplicated pS2 protein and is produced in large amounts by the exocrine pancreas, and has been shown to have spasmolytic and gastric acid secretion inhibitory effects (Jorgensen et al., 1982). The mouse (*mSP*) and the human (*hSP*) counterparts of *PSP* have been cloned, and found to be expressed within normal stomach mucosal cells (Tomasetto et al., 1990). Moreover, three pS2 similar proteins, xP1, xP2 and xP4, have been identified in *Xenopus*, and are expressed in the stomach mucosa (xP1, xP4) (Hauser and Hoffman 1991), and in the skin (xP2) (Hauser et al., 1992). Recently, a new member of the family, intestinal trefoil factor (ITF), has been identified in rat intestinal mucosa (Suemori et al., 1991). All the members of this family of proteins exhibit one (pS2, xP1, ITF), two (PSP, mSP, hSP, xP2), or four copies (xP4) of a domain containing strikingly conserved cysteine residues, named the P-domain (Tomasetto et al., 1990), that is presumed to participate in a trefoil disulphide loop structure (Thim, 1989; Carr, 1992) and to contribute to the protease resistance of these proteins. In fact, PSP has been shown to be resistant to a number of proteases, despite the presence of multiple potential proteolytic sites, and to exert biological activity when given by an oral route to animals (Jorgensen et al., 1982). All of these results suggest that the P-domain peptides could be involved in the maintenance of mucosal integrity (Suemori et al., 1991). This action may result from a growth factor-like role of the P-domain peptides, a role previously proposed for pS2 (Jakowlev et al., 1984) based on structural similarities with such factors.

To investigate further the biological function of the pS2 protein, we have now cloned the mouse *pS2* cDNA, and analyzed the physiological pattern of *pS2* gene expression in this animal, particularly in the mammary gland during postnatal development and in perinatal and adult gastrointestinal tract. Our results show that the pS2 protein sequence and expression pattern are well conserved between human and mouse. No pS2 mRNA was found in mammary gland samples, but the mouse *pS2* gene is physiologically expressed in mucosal cells of the stomach where it can be detected from the 17th day of gestation. Comparison of the *pS2* and *mSP* gene patterns of expression, by in situ analysis on serial sections, reveals that the one P-domain (pS2) and the two P-domain (mSP) peptides are specifically expressed in different tissues and/or at different cellular levels in the mouse gastrointestinal tract showing spatial complementarity.

Materials and Methods

Tissue Collection

Part of the tissue samples were immediately frozen in liquid nitrogen for RNA extraction. The remainder of the tissue was conserved in formol before inclusion in paraffin for histological examination and in situ hybridization. Perinatal gastrointestinal tissues (collected daily from 14 d of gestation to 3 d after birth, and 7, 14, and 21 d postnatally) were dissected under the binocular microscope, and immediately fixed in formol.

Mammary glands were surgically excised from virgin mice (before and after sexual maturity), pregnant mice at various stages of gestation, lactating mice (when the lobulo-alveolar development was complete), and after weaning (during involution of the lobulo-alveolar structures).

Isolation of RNA and Northern Hybridization

RNA was prepared by a single-step method by acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski and Sacchi, 1987). RNAs were fractionated by gel agarose electrophoresis (1%) in the presence of formaldehyde and transferred to nylon membranes (hybond N; Amersham International, Amersham, UK). Filters were acidified (10 min, 5% CH3-COOH) and stained (10 min, 0.004% methylene blue, 0.5 M CH3COONa, pH 5.0) before hybridization. Double-stranded DNA was ^{32}P-labeled by random-priming (Feinberg and Vogelstein, 1983). Northern blots were hybridized for 18 h under stringent conditions (50% formamide, 42°C). Washings were performed in 2× SSC, 0.1% SDS at 22°C, followed by 0.1× SSC, 0.1% SDS at 55°C.

cDNA Synthesis

1 μg of poly(A)$^+$ RNA, purified by oligo(dT)-cellulose chromatography from mouse stomach total RNA, was heated at 65°C for 3 min, in 16.5 μl of water, quenched on ice, added to 2 μl of 10X RTC buffer (1× = 50 mM Tris-HCL, pH 8.15, at 41°C, 6 mM $MgCl_2$, 40 mM KCl, 1 mM DTT, each dNTP at 1.5 mM), 0.25 μl (10 U) of RNasin (Promega Biotec, Madison, WI), 0.5 μl of EcoRI adaptor-(dT)18 primer (5'CCCTCGAGGATCCGA-ATTC18X(dT)3') (1μg/μl), and 10 U of AMV-reverse transcriptase (avian myeloblastosis virus), and incubated for 1.5 h at 42°C. The reaction mixture was diluted to 1 ml with TE buffer (10 mn Tris-HCl, pH 7.5, 1 mM EDTA) and stored at 4°C.

Amplification of 3' End cDNA Sequences Related to P-Domain Peptides

Comparison of human pS2, mSP, and hSP cDNA sequences allowed us to define an oligonucleotide 5'CCGAATTCGGATCCCTCAACTGTGGCT-TCCCGGGC3', capable of identifying the coding region of the mouse *pS2* gene. It was constituted by 18 highly conserved nucleotides, located in the 5' end of the P-domain sequence (Tomasetto et al., 1990). Recognition sequence for EcoRI endonuclease was added to create a restriction site at the 5' end of the oligonucleotide. Using this specific adaptor–primer and the nonspecific adaptor-oligo(dT) primer, cyclic thermal amplification with Taq-polymerase (Perkin-Elmer Corp., Norwalk, CT) was according to Frohman et al. (1988). PCR reactions were performed in a final volume of 50 μl covered with 100 μl mineral oil. Reaction included 5 μl of 10× PCR buffer (Tris-HCl 100 mM, pH 8.3; KCl 500 mM; $MgCl_2$ 15 mM; Gelatin 0.01%), 250 μM of each dNTP, 0.5 μM of the 5' amp specific adaptor-primer, 0.5 μM of the 3' amp adaptor-oligo(dT) primer and 2.5 U of Cetus Taq polymerase. Initial denaturation was for 5 min at 95°C, annealed for 2 min at 50°C and then the cDNA was extended for 40 min at 72°C. Amplification was for 40 cycles (40 s at 94°C, 2 min at 50°C, 3 min at 72°C) followed by 15 min final extension at 72°C. 10 μl of the PCR product were analyzed on 1% agarose gel containing ethidium bromide at 0.5 μg/ml. Material from amplification (~250–500 bp) was extracted after electrophoresis on a low melting point agarose gel (GIBCO BRL, Gaithersburg, MD), EcoRI digested and cloned in the Bluescript vector (SK$^+$; Stratagene Ltd., Cambridge, UK), so as to obtain a "mini-library" enriched in P-domain peptide sequences. After transfection in XL1Blue bacteria, the library was plated on 6 petri dishes, at low density (40 colonies per petri dish), and replicated on nylon filters (Biodyne A; Pall Corporation, Glen Cove, NY). 160 recombinants were first hybridized with a ^{32}P-labeled *mSP* probe (Tomasetto et al., 1990), at 42°C in the presence of 50% formamide. Washings were performed in 2× SSC, 0.1% SDS at 22°C, followed by 0.1× SSC, 0.1% SDS at 55°C. After autoradiography, the remainding negative clones (only 10) were sequenced by the dideoxy protocol employing Sequenase and dGTP analogs. The same conditions of hybridization were used to screen the lambda GT10 mouse stomach library with the previously isolated cDNA and to sequence the positive clones, amplified after subcloning in the bluescript vector.

In situ Hybridization

Deparaffined and acid-treated sections (6 μm thick) were treated with proteinase K and hybridized overnight with ^{35}S-labeled antisense transcripts

1. *Abbreviations used in this paper*: hSP, human spasmolytic polypeptide; mSP, mouse spasmolytic polypeptide; PSP, porcine spasmolytic polypeptide.

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CCATGGAGCACAAGGTGATCTGTGTCCTCGCTGTGGTCCTCATGCTGGCCTTCGGCAGTCTTGCCCAGGCCCAG   74
  M  E  H  K  V  I  C  V  L  A  V  V  L  M  L  A  F  G  S  L  A↑Q  A  Q     24

GCCCAGGCCCAGGCCCAGGAAGAAACATGTATCATGGCCCCCCGGGAGAGGATAATTGTGGCTTCCCCGGTGTC   149
 A↑Q  A  Q  A  Q  E  E  T  C  I  M  A  P  R  E  R  I  N  C  G  F  P  G  V     49

ACCGCCCAGCAGTGCACGGAGAGAGGTTGCTGTTTTGATGACAGTGTCCGGGGATTCCCGTGGTGCTTCCACCCC   224
 T  A  Q  Q  C  T  E  R  G  C  C  F  D  D  S  V  R  G  F  P  W  C  F  H  P     74

ATGGCCATCGAGAACACTCAAGAAGAAGAATGTCCCTTCTAAGGTCCATCCTGAGAGAACTGGCTACATCAAGAC   329
 M  A  I  E  N  T  Q  E  E  E  C  P  F  *                                     88

TTGGCACCCTCCACCTGGGCACTGGAGCCACCTGGCCCACCTGCTACATACACACCTATTCTGTGGCTGGATCTG   374

GCTGGTGACACAGTTCAACCCCTCAGACTTTTAGTCCTCGAATTCGGCCTGAGAATTAAAAGAGATGAATGTTAA   449

AAAAAAAAAAAAAA                                                               464
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Figure 1. Mouse *pS2* cDNA and its deduced amino acid sequence. Nucleotide residues are numbered in the 5′ to 3′ direction and amino acids in the open reading frame are designated by the one letter code. The 5′AATTGTGGCTTCCCCGGT3′ sequence corresponding to the P-domain specific primer is underlined. Arrows indicate the two putative signal peptidase sites (Von Heijne, 1986). These sequence data are available from EMBL/GenBank/DDBJ under accession number Z21858.

from a *pS2*- or a *mSP*-cDNA insert, subcloned in Bluescript II (Stratagene). Hybridization was followed by RNase treatment (20 μg /ml, 30 min, 37°C) and two stringent washings (2× SSC, 50% formamide, 60°C, 2 h), before autoradiography using NTB2 emulsion (Eastman Kodak Co., Rochester, NY). Autoradiography was for 36 h to 4 d.

Results

Identification of Mouse pS2 Coding Sequences

Assuming that the physiological expression of the *pS2* gene in the stomach may be conserved between human and mouse, a cDNA library, enriched in P-domain peptide sequences, was constructed from mouse stomach tissue. mRNAs isolated from mouse stomach were reverse transcribed and amplified by PCR using an EcoRI adaptor-oligo(dT)primer and an EcoRI adaptor-P-domain specific primer, deduced by comparison of published sequences of human *pS2*, *mSP* and *hSP* (see Materials and Methods). 250–500 bp-long cDNA PCR products, were cloned at the EcoRI site of a bluescript vector after elution from low melting gel. 160 recombinant clones were first screened with a ^{32}P-labeled *mSP* probe to eliminate all clones containing sequences related to the *mSP* gene (150 clones). The ten remaining clones were sequenced. One of them, containing a 245-bp insert, corresponded to a putative 45-amino acid sequence, closely related (67% homology) to the COOH-terminal part of the human pS2 protein. This cDNA was used to probe a lambda GT10 mouse stomach cDNA library (Tomasetto et al., 1990) to isolate the corresponding full-length cDNA. 20 positive clones were obtained; three of them were subcloned into a bluescript vector and sequenced, yielding a potential mouse *pS2* cDNA of 464 bp in length (Fig. 1).

The Mouse and Human pS2 Protein Sequences Are Highly Homologous

The protein sequence, as deduced from the cDNA sequence (Fig. 1), was 87 amino acid residues in length and contained a hydrophobic NH_2-terminal sequence corresponding to a putative signal peptide of either 21 or 25 residues, according to possible cleavage sites (Von Heijne, 1986). The putative mature protein, made up of either 62 or 66 amino acids, showed a mean similarity of 67 and 47% with the human and *Xenopus* (xP1) pS2 proteins, respectively, and an absolute conservation of the six consensus cysteine residues present in the P-domain (Fig. 2). Moreover, 49 and 39% similarity were also observed with the first and the second P-domains of mSP, respectively (Fig. 2). The highest similarity was with the human pS2 protein suggesting that the cloned cDNA corresponded indeed to the mouse *pS2* cDNA.

In addition to the conservation of the consensus cysteine residues, a strong similarity was also seen in residues adjacent to the sites of presumed intrachain disulfide bond formation (Fig.2, consensus sequence). Moreover, the last six COOH-terminal amino acids of the mouse pS2 protein, showed 83% similarity with the human peptide sequence. Interestingly, this part of the pS2 protein corresponds to the third exon of the human *pS2* gene, and, of all members of the P-domain family, only the mouse and human pS2 possess this acidic motif. Independently of the cleavage site of the signal peptidase, the secreted mature form of the mouse pS2 protein, contains a glutamine at its NH_2-terminal end. This NH_2-terminal residue was already observed in PSP, xP1, and xP2, whereas human pS2, mSP, hSP and xP4 display an NH_2-terminal glutamic acid. Both glutamic acid and glutamine residues could be enzymatically changed into a pyroglutamic acid residue, as previously observed for pS2 in gastric juice (Rio et al., 1988a) and PSP (Thim et al., 1985), suggesting that the same posttranslational modification could exist in the mouse pS2 peptide.

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m pS2   MEHKVICVLAVVLMLAFGSLAQAQAQAQEET C .IMAPRE R I NCG FP G VTAQQ C TERG CCF DDSVRGF PWCF H P MAIENTQEEEQPF
                                                                                                  ****  *
h pS2   MAT--N----A-VL-S---L-T--E--T.....--- - .TV---- - Q --- -- - --PS- - ANK- ---- --T---V ---- Y - NT-DVPP----E-
xP1     -NY--F-..L-AIA-IV--IGS-NG--AFT--Q - .SVERLA - V --- YS - I-P-E - -KQ- ---- -STIQDA ---- Y - R-TPEY
mSP     RPRGAPL---L-V-..-.-HALVEGEKPSPCR - SRLT-HN - K --- -- - I-SE- - FDL- ---- -S--A-V ---- - - LPNQESEQ
                                         - .V-EVSA - K --- Y- - ISPED - AS-N --- SNLIFEV ---- F - QSV-DCHY

CONSENSUS   C ------- R - NCG FP/YS G IT/VS--- C ---G/N CCF D/S--V/I--- PWCF - P
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Figure 2. Sequence conservation between the mouse (*mpS2*), human (*hpS2*) and *Xenopus* (*xP1*) one P-domain peptide, and the murine (*mSP*) two P-domain peptide. Amino acid sequences determined from cDNA sequence data have been aligned. Gaps (.) have been introduced into the sequences to obtain maximal alignment of identical amino acids. The complete one letter code sequence is given for mpS2; in the sequence of hpS2, xP1, and mSP, only nonconserved amino acids are indicated; (-) represents the conserved amino acids. Invariant amino acids, observed in the four sequences, are enclosed in boxes. Stars indicate the acidic COOH-terminal part, specific to the mouse and human pS2 proteins. Arrows indicate the putative signal peptidase sites (Von Heijne, 1986), whereas the arrowhead indicates the defined signal peptidase site (Rio et al., 1988*a*). Consensus sequence established from the four sequences is also given.

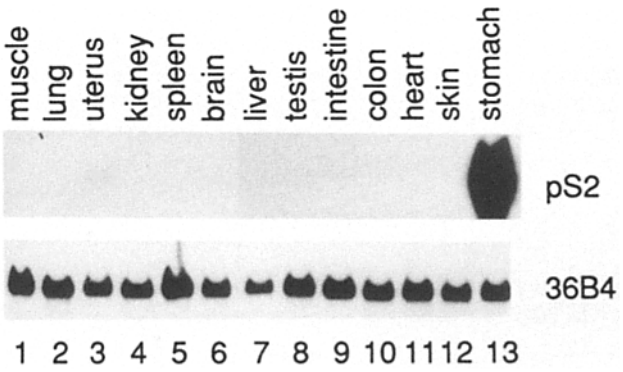

Figure 3. Northern blot analysis of normal mouse tissue RNA. Each lane contained 10 μg of total RNA. From left to right: muscle, lung, uterus, kidney, spleen, brain, liver, testis, small intestine, colon, heart, skin, and stomach. A 600-base-long pS2 transcript was detected in lanes *3* and *13*, corresponding to uterus and stomach. The *36B4* probe (Masiakowski et al., 1982) was used as positive internal control. Autoradiography was overnight for *36B4* hybridization and *pS2* hybridization in the stomach (lane *13*), whereas *pS2* hybridization of all other samples are exposed for 2 d.

The pS2 Gene Is Highly Expressed in Mouse Stomach

Northern blot analysis of total RNA from a panel of normal mouse tissues, using a ^{32}P-labeled mouse *pS2* cDNA corresponding to the complete coding sequence, revealed a single pS2 transcript of 600 bases in the stomach (Fig. 3, lane *13*), whereas muscle, lung, kidney, spleen, brain, liver, testis, small intestine, colon, heart, skin (Fig. 3, lane *1*, *2*, and *4–12*), and pancreas (data not shown) were negative. An unexpected, very weak response was also observed in a mouse uterine sample (Fig. 3, lane *3*). Since the normal human uterus does not express the *pS2* gene (Rio et al., 1987; Henry et al., 1991*a*; Piggot et al., 1991), we cannot exclude that this low hybridization signal resulted from cross-reactivity between the mouse *pS2* probe and another member of the P-domain family which may be present in the uterus. Further study should resolve this question.

Human and mouse mammary gland undergo most of their morphogenetic and functional changes postnatally, under hormonal and growth factor control, via similar processes (Topper and Freeman, 1980; Vonderhaar 1988; Coleman et al., 1988; Robinson et al., 1991). As the expression of the *pS2* gene is dependent on oestradiol in tumoral human mammary epithelial cells (Rio et al., 1987; Berry et al., 1989; Nunez et al., 1989; Henry et al., 1989; Wysocki et al., 1990), we wondered whether this expression may be reminiscent of a physiological expression occuring during these processes. Thus, the expression of the *pS2* gene was investigated during the physiological processes of proliferation, differentiation and postweaning involution which occur in the normal mouse mammary gland. Using Northern blot and in situ analysis, no pS2 mRNA was observed in the mouse mammary gland from virgin (before and after reaching sexual maturity), pregnant, lactating, or postlactating mice (data not shown).

pS2 and mSP Genes Exhibit Specific Patterns of Expression in the Adult Mouse Gastrointestinal Tract

The *pS2* and *mSP* patterns of expression were investigated in the entire mouse gastrointestinal tract in the adult animal. In situ hybridization, using ^{35}S-labeled pS2 and mSP antisense RNA probes was performed on paraffin embedded tissues. pS2 mRNA was expressed in the gastric mucous cells lining the surface epithelium and the pits, regardless of the portion of the stomach considered: fundus (Fig. 4, *A* and *B*), antrum (Fig. 4, *E* and *F*) and antrum-pyloric (Fig. 5, *A* and *B*) regions. In contrast, the mSP mRNA was observed in cells confined to the junction between the pits and the base of the glands within the fundic mucosa, corresponding presumably to the mucous neck cells (Fig. 4, *C* and *D*), and in the base of the antrum (Fig. 4, *G* and *H*) and antrum-pyloric (Fig. 5, *C* and *D*) glands. Thus, although they are both observed in the fundus (Fig. 4, *A–D*), the antrum (Fig. 4, *E–H*) and the antrum-pylor (Fig. 5, *A–D*), pS2 and mSP mRNAs showed a striking difference in their cellular localization, resulting in a complementary pattern of expression.

In the small intestine, pS2 mRNA could not be detected while the *mSP* gene was strongly expressed within the Brunner's glands confined to the proximal duodenum (Fig. 5, *C* and *D*). Neither the pS2 nor the mSP mRNAs were observed in the large intestine (data not shown).

pS2 is Transiently Coexpressed with mSP within the Brunner's Glands during Perinatal Development of the Mouse Gastrointestinal Tract

In situ hybridization analysis revealed that both *pS2* and *mSP* genes are expressed during the perinatal development of the gastrointestinal tract. pS2 mRNA was first observed at day 17 in embryonic stomach, and, at day 18, both pS2 and mSP mRNAs were detected in the epithelial cells lining the surface and the nascent pits and glands, although mSP mRNA levels were less intense than those of pS2 mRNA (data not shown). From birth onwards, these patterns of expression progressively evolved and a clearcut pattern of pS2 and mSP expression, as observed in adult, became apparent within the stomach at postnatal day 14.

In addition, 18-d embryos showed expression of both mRNAs in the glands of the gastro–duodenal junction corresponding partly to the developing Brunner's glands (data not shown). From birth onwards, the Brunner's glands, localized at the lower intervillus area of the proximal duodenum, were strongly stained with the mSP probe (Fig. 5, *G* and *H*) and showed a transient co-expression of pS2 (Fig. 5, *E* and *F*), no longer observed postnatal day 14. *pS2* and *mSP* genes were never expressed in the developing large intestine (data not shown).

Discussion

We have identified a mouse homolog of the human *pS2* cDNA. The putative mouse pS2 protein is well conserved, exhibiting notably a typical P-domain (Tomasetto et al., 1990) and a COOH-terminal acidic motif, previously described only in the human pS2 protein. The mouse *pS2* gene is expressed in the fundic, the antral, and the antrum-pyloric stomach mucosal cells, but not in the normal mammary gland. Moreover, a comparative in situ analysis of the one

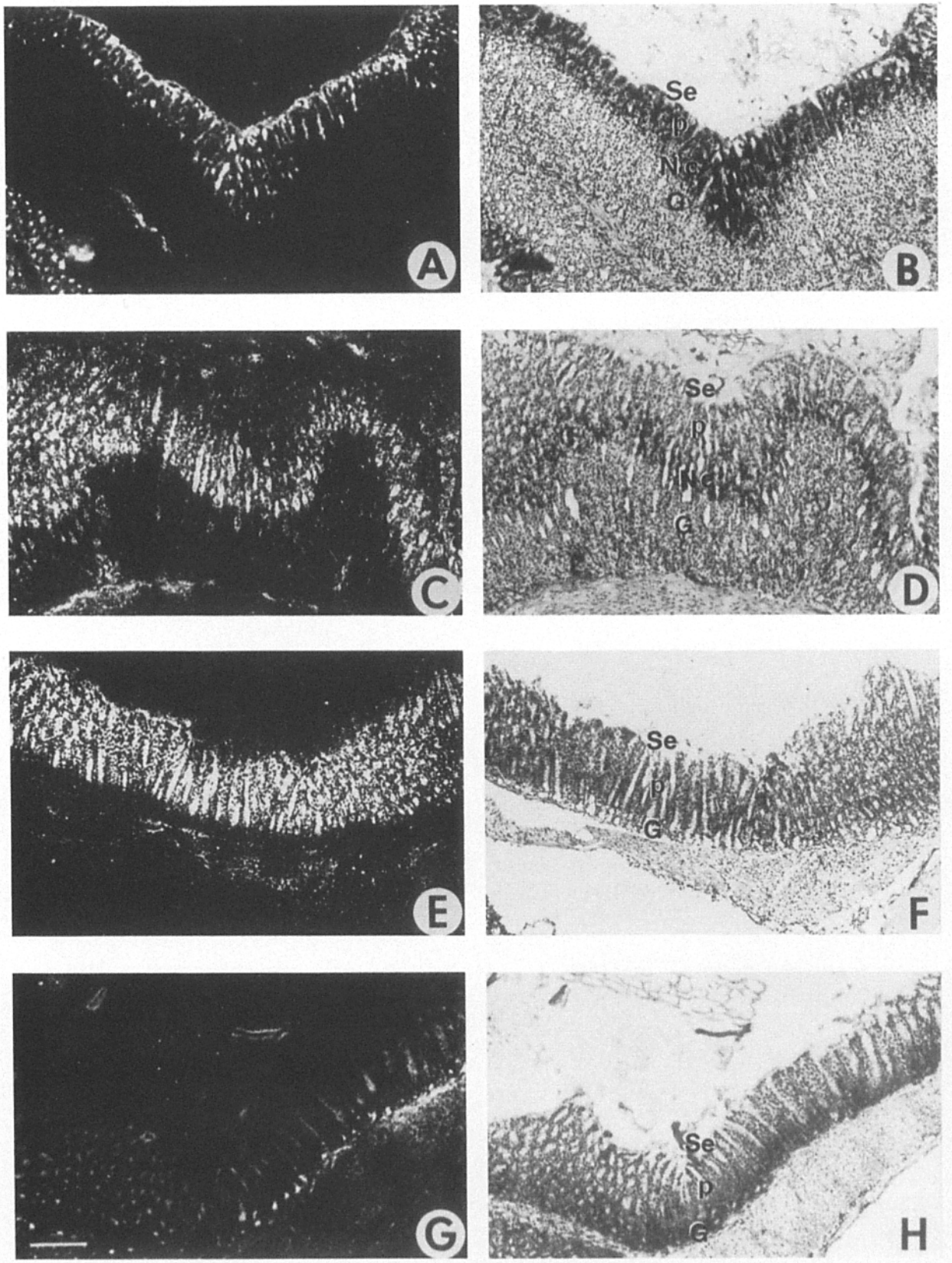

Figure 4. In situ hybridization of pS2 and mSP mRNA in adult mouse stomach. Sections from the fundus (*A–D*) and the antrum (*E–H*) were hybridized with antisense probes specific for mouse pS2 (*A*, *B*, and *E*, *F*) and mSP (*C*, *D*, and *G*, *H*). Both parts of the stomach expressed *pS2* and *mSP*, showing a complementary pattern of expression; (*A–D*) Fundus, pS2 mRNA in the surface epithelium (*Se*) and the pits (*p*), mSP mRNA in the neck cells (*Nc*); (*E–H*) antrum, pS2 mRNA in the surface epithelium (*Se*) and in the pits (*p*), mSP in the base of the glands (*G*). No significant labeling above background was found when using sense mouse pS2 and mSP RNA probes (data not shown). Dark field: (*A*, *C*, *E*, and *G*) and bright field: (*B*, *D*, *F*, and *H*) of the same sections. Bar, 160 μm.

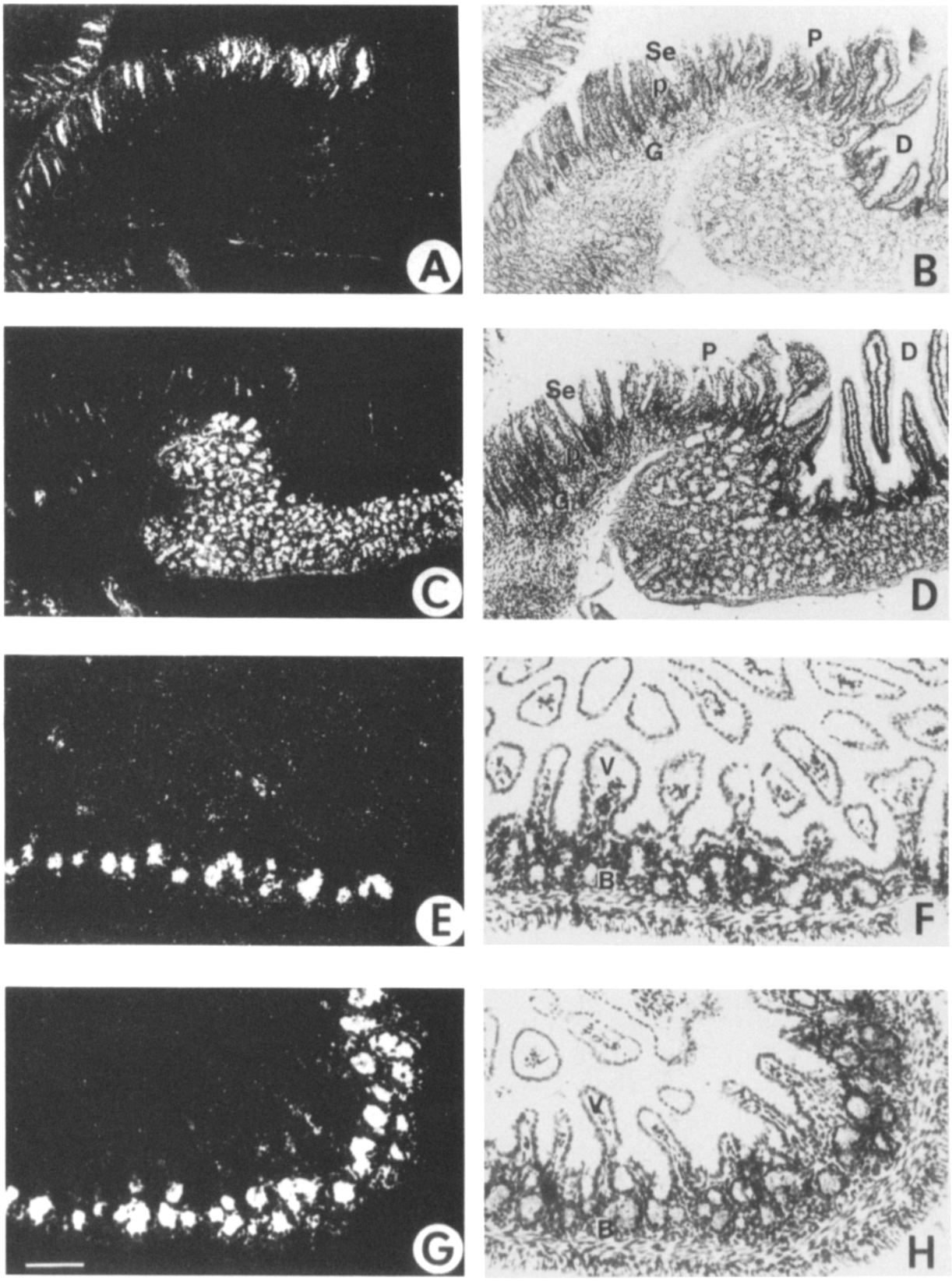

Figure 5. In situ hybridization of pS2 and mSP mRNA in adult (*A–D*) and perinatal (*E–H*) mouse duodenum. (*A* and *B*) *pS2* expression in the surface epithelium (*Se*) and the pits (*p*) of the pylor (*P*); (*C* and *D*) *mSP* expression in the Brunner's glands of the proximal part of the duodenum (*D*). (*E–H*) presence of both pS2 (*E* and *F*) and mSP (*G* and *H*) mRNAs in the Brunner's gland of the duodenum from a new-born mouse, on the day of birth. The villi (*V*) are negative. No significant labeling above background was found when using sense mouse pS2 and mSP RNA probes (data not shown). Dark field (*A*, *C*, *E*, and *G*) and bright field (*B*, *D*, *F*, and *H*) of the same sections. Bars: (*A–D*) 160 μm; (*E–H*) 80 μm.

P-domain (pS2) and the similar two P-domain (mSP) RNAs on the whole mouse gastrointestinal tract showed a distinct pattern of expression for the two genes. The *pS2* gene is expressed in cells located within the surface and the pits, while the mSP-expressing cells are the fundic neck cells and basal cells of the antral and pyloric glands. A previous analysis, limited to the presumed normal human antrum, showed a coexpression of the *pS2* and *hSP* genes, in the superficial epithelium (Tomasetto et al., 1990). However, in accordance with the present study, no PSP immunoreactivity was seen in the surface epithelium and in the pits of the pig stomach (Rasmussen et al., 1992). Interestingly, a differential cellular localization of *pS2* and *hSP* gene expression, similar to that described here, has already been observed in the ulceration-associated cell lineage (UACL) in damaged human small intestine (Wright et al., 1990). This complementary pattern of expression suggests that, although they may have similar function (Thim, 1988; Baker, 1988), one P-domain and two P-domain peptides could act on different regions of the gastrointestinal tract, perhaps locally, in an autocrine manner.

During the perinatal development, *pS2* and *mSP* are already coexpressed in immature stomach and duodenum of 18-d mouse embryos. Adult-like pS2 and mSP pattern of expression progressively develops during the two first weeks of life, concomitantly with the neonate differentiation of the glands (Ouellette and Cordell, 1988; Dial and Lichtenberger, 1989). At 14 d, the *pS2* gene is no longer expressed in the duodenal Brunner's glands.

In human, the oestradiol-regulated *pS2* gene expression represents a discriminating marker for hormonal status of the breast tumors (Rio et al., 1987; Foekens et al., 1990; Henry et al., 1991; Schwartz et al., 1991; Klijn, 1992). Either no *pS2* expression (Rio et al., 1987; Skilton et al., 1989) or a weak *pS2* expression (Piggott et al., 1991; Hähnel et al., 1991; Predine et al., 1992) has been observed in the normal human mammary gland. Assuming that the regulation of the *pS2* gene is conserved between human and mouse, the absence of *pS2* gene expression during the mouse mammary gland postnatal development, even at steps depending on oestrogens and/or EGF (Coleman et al., 1988; Vonderhaar, 1988; Snedeker et al., 1991), suggests that the expression of *pS2* in human breast carcinomas, does not represent an inappropriate physiological expression. Moreover, preliminary analysis of mouse mammary tumors showed no expression of the *pS2* gene (data not shown).

The biological function of the pS2 protein remains unknown. In addition to its normal expression in the stomach, the human *pS2* gene is overexpressed in acute inflammatory disorders of the gastrointestinal tract (Wright et al., 1990; Rio et al., 1991) and in a panel of carcinomas (Rio et al., 1987; Luqmani et al., 1989; Welter et al., 1991; Henry et al., 1991*a*; Seitz et al., 1991). Growth modulating activity, protease inhibitory function or gastric mucosa protective effect, have been proposed (Jakowlev et al., 1984; Baker, 1988; Thim, 1989). Due to the large spectrum of possible regulating pathways (Berry et al., 1989; Nunez et al., 1989) and expression pattern of the *pS2* gene (Rio and Chambon, 1990; and references therein), one may imagine that the pS2 protein possesses various tissue specific functions. Nevertheless, an overall concept could be proposed. The human and mouse *pS2* genes are always expressed by mucin-expressing cells, and more precisely by MUC1-expressing tissues, i.e., the stomach (Luqmani et al., 1989), the UACL (Wright et al., 1990*b*), the mammary gland or the pancreas (Lan et al., 1990; and for review see Spicer et al., 1991). Mucins are glycoproteins which act as a barrier between the cell membrane and the external environment (for review see Devine and McKenzie, 1992 and references therein). Interestingly, MUC1 (previously referred to as the polymorphic epithelial mucin) is mainly associated with breast, ovarian, and pancreatic tumors, where it is over-expressed and/or altered, notably in its carbohydrate structures (Hilkens, 1988; Merlo et al., 1989; McKenzie and Xing, 1990; Ceriani et al., 1992). Thus, there is a relationship between the pS2-expressing tissues, and it is tempting to speculate that the pS2 protein biological function is related to the MUC1 subtype of mucins. In this context, it has already been proposed that the pS2 protein may play a role in the degradation of the carbohydrate and of the tumoral extracellular glycoproteins (Tomasetto et al., 1990).

Finally, due to their similar sequence and pattern of expression, a comparable function can be expected for the human and mouse pS2 proteins. The knowledge of the mouse homolog of the *pS2* cDNA provides the basis for systematic molecular biological research on animal models. The physiological function of the pS2 protein will be investigated in transgenic mice, through gene disruption in embryonic stem cells. More applied studies should also be initiated to establish the precise participation and the possible clinical implications of the pS2 protein in gastrointestinal pathological processes.

We thank Drs. P. Basset and P. Simpson for critical reading of the manuscript, and C. Arnold, M. Loriot, and C. Wendling, for technical assistance.

This work was supported by funds from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Ministère de la Recherche et de l'Enseignement Supérieur, the Association pour la Recherche sur le cancer, the Fondation pour la Recherche sur le Cancer, the Ligue Nationale contre le Cancer, the Centre Hospitalier Universitaire Régional, and the Fondation Jeantet. C. Tomasetto is a recipient of an Isabelle Oberlé Fellowship.

Received for publication 29 December 1992 and in revised form 25 March 1993.

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