

# **The progesterone receptor in human term amniochorion and placenta is isoform C.**

by

Anthony H Taylor, Penny C McParland, David J Taylor & Stephen C. Bell.

from

*Preterm Birth Research Group, Reproductive Sciences, Department of Cancer Studies and Molecular Medicine, University of Leicester, Leicester, Leicestershire, LE2 7LX, UK.*

Corresponding author:

Anthony Taylor

Preterm Birth Research Group Lecturer

Reproductive Sciences Section

Department of Cancer Studies and Molecular Medicine

University of Leicester

P.O. Box 65

Leicester

Leicestershire

LE3 7LX

Tel: +44 (0)116 252 5822

Fax: +44 (0)116 252 5846

e-mail: [aht3@le.ac.uk](mailto:aht3@le.ac.uk)

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## **Abstract**

The mechanism that initiates human parturition has been proposed to be 'functional progesterone withdrawal' whereby the 116-kDa B-isoform of the progesterone receptor (PR-B) switches in favour of the 94-kDa A-isoform (PR-A) in reproductive tissues. Recently, other PR isoforms, PR-S, PR-C and PR-M generated from the same gene have been identified and partially characterised. Using immunohistochemical, western blotting and RT-PCR techniques, evidence is provided that the major PR isoform present in human term fetal membranes (amnion and chorion) and syncytiotrophoblast of the placenta is neither of the classical nuclear PR-B or PR-A isoforms but is the N-terminally truncated 60 kDa PR-C isoform. Evidence is also provided that the PR-C isoform resides in the cytoplasm of the expressing cell types. Data are also presented to show that PR-B, PR-A and PR-S isoforms are essentially absent from the amnion and chorion, whereas PR isoforms A, B, C and S are all present in the decidua, with PR-A being the major isoform. The syncytiotrophoblast of the placenta contains the cytoplasmic PR-C isoform, but not PR-A, PR-B or PR-S. The major PR isoform in the amnion, chorion and placenta is PR-C, suggesting that the cytoplasmic PR-C isoform has a specific role in extra-embryonic tissues and may be involved in the regulation of human parturition.

## Introduction

Progesterone receptors are members of a superfamily of ligand-activated nuclear transcription factors comprised of specific domains involved in DNA binding, hormone binding, and transactivation (1). Progesterone activation of PR in target tissues is mediated via dimerisation and phosphorylation of the receptor, resulting in binding to *cis*-acting progesterone response elements on DNA and the modulation of the promoters of target genes (1, 2). The human PR-A isoform differs from the PR-B isoform in lacking the first 164 amino acids contained in PR-B (3). Both are translated from distinct mRNA transcripts generated from a single gene under the control of separate oestrogen sensitive promoters (4). Previous work has identified three additional AUG translation sites with a possible methionine site at 595 that is predicted to generate a protein of approximately 60 kDa (5).

Although much work has been performed on PR-B and PR-A, little work has been undertaken on the other 7 transcripts generated from the PR gene (5), despite there being evidence that some of these are translated into functional 38 kDa, 60kDa, 71kDa or 78kDa proteins in malignant progesterone target tissues (6) and that these are coordinately up-regulated by oestrogens and down-regulated by progestins (7, 8). Evidence also exists for other PR isoform such as PR-C, PR-S and PR-T, which could be genomic mediators of progestin action (9, 10) and for three membrane progestin receptors that are classical G-coupled protein receptor-transduction molecules first identified in the teleost oocyte called mPR $\alpha$ , mPR $\beta$  and mPR $\gamma$  (11, 12).

Progesterone receptors have been proposed to play a key role in the control of human labour and parturition whereby the levels of the PR-B isoform, which is often considered to be the dominant isoform, fall during labour leaving the PR-A isoform as the predominant form leading to a 'functional progesterone receptor withdrawal' (13).

Evidence to support this occurring in the uterine myometrium exists (14). In other human reproductive tissues, such as the decidua, ovary and the oviduct (15, 16), PR-A appears to be the predominant progestin regulator with PR-B maintaining a supporting role suggesting that progestin signalling in the human uterus at the end of parturition is far more complex than a PR-B to PR-A isoform switching mechanism (14). Despite  
30 there being a paucity of data to support 'functional progesterone receptor withdrawal' in tissues at the fetal-maternal interface, i.e. in the fetal membranes, decidua and placenta, many still consider that only the PR-B and PR-A isoforms are present (17, 18).

Recent data has suggested that at least five nuclear PR-isoforms are present in the  
35 human decidua and that all five isoforms are decreased after labour (19). However, although western blotting techniques also indicated the presence of several PR isoforms in amniotic nuclear extracts, immunohistochemical methods failed to detect any PR isoforms in the amnion and chorion (19).

In the present study examining the pattern of expression of PR isoforms in human  
40 fetal membranes (amnion and chorion), decidua and placenta at term, we demonstrate that the major isoform present in the fetal membranes and placenta is a cytoplasmic 60-kDa PR-C isoform, that PR-B or PR-A is not expressed in the amnion or chorion, and the 94kDa PR-A protein is the dominant PR isoform in the decidua

## Materials and Methods

### 45 Patient Samples.

Local Research Ethics Committee approval for the study was obtained and all patients signed informed consent for their tissues to be used. Fetal membranes and placenta (n=6) were collected from term patients undergoing elective Caesarean section prior to labour. All tissues were divided into 3 parts; one fixed in formalin and embedded in  
50 paraffin for histological examination, the other two snap-frozen in liquid nitrogen and stored at -80°C for later RNA and protein extraction. Enriched amnion was obtained carefully peeling of the amnion from chorion and decidua. Enriched decidua was obtained using the edge of a microscope slide to carefully remove a thin layer from the inverted fetal membranes. Enriched chorion was obtained by using the edge of the  
55 microscope slide to scrape away the remaining decidua. A 2cm<sup>3</sup> block of placenta from the mid-part of a cotyledon was taken and washed briefly with sterile PBS before division into 3 parts.

### Immunohistochemistry.

Five µm sections of tissue dried for 48 hr prior to de-waxing and re-hydration through  
60 graded alcohol to H<sub>2</sub>O were subjected to boiling 10mM citric acid for 12min followed by cooling for exactly 20min before transfer into cold H<sub>2</sub>O to retrieve antigenic sites. Endogenous peroxidase activity and specific binding was blocked with 6% H<sub>2</sub>O<sub>2</sub> for 15 min, followed by incubation with non-immune rabbit serum (10% in PBS) for 30 min, respectively. Endogenous avidin and biotin sites were blocked with Avidin-Binding  
65 blocking solutions (Vector Laboratories, Peterborough, UK) according to the manufacturer's instructions. Primary antibody, PR clones 1A6, San27, clone 16, or the polyclonal antibody C-20 (Santa Cruz SC-539) were applied at the indicated concentrations (Fig 1) in 10% non-immune rabbit serum or 10% swine serum overnight

at 4°C. In some studies, C-20 antibodies were pre-incubated (3 hr at room temperature)  
70 with a 7-fold excess of immunising peptide before use. After copious washing in PBS,  
biotinylated rabbit anti-mouse or swine anti-rabbit secondary antibodies (1:400) were  
applied for 1hr 30 min, the sections washed and avidin-biotin complexes applied for 30  
min. Colour was developed over antigenic sites using 3, 3'-diaminobenzidine for 5 min.  
After copious washes in dH<sub>2</sub>O, sections were lightly counterstained with haematoxylin,  
75 dehydrated through graded alcohols, cleared in xylene and permanently mounted in  
XAM (BDH, Poole, UK). Photomicrographs were obtained at the indicated  
magnifications on a Zeiss Axioplan compound microscope fitted with a Nikon DN-100  
digital camera. Images were digitally enhanced using proprietary software.

#### **RNA preparation and RT-PCR.**

80 Total cellular RNA was obtained from 100mg of tissue using Trizol reagent (Invitrogen,  
Paisley, UK) according the manufacturer's instructions, and genomic DNA  
contamination removed by treating the samples with RNase-free DNase 1 (Promega,  
Southampton, UK) for 1 hour at 37°C, followed by phenol-chloroform extraction and  
isopropanol precipitation. After verification of RNA quality by UV-spectrophotometer  
85 analysis and agarose gel electrophoresis, one µg of RNA was reverse transcribed using  
AMV-RT for one hr at 42°C. A minus RT control was obtained by substituting DEPC-  
treated water for the AMV-RT enzyme.

PCR was performed using PR primer combinations that identified all PR isoforms, the  
PR-B isoform, the PR-A and PR-B isoforms or the PR-S isoform (20); (Table 1).

#### **90 Western Blotting.**

Protein extracts were obtained by homogenising samples in ice-cold lysis buffer (1%  
Ipegal, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 2mM  
EDTA, 50mM sodium fluoride, 1 mM sodium metavanadate, 0.2 mg/ml aprotinin all

from Sigma-Aldrich, Poole UK, in PBS (pH 7.0) but without phenyl methyl sulphonyl  
95 fluoride) (21). After incubation on ice for 30 min, insoluble material was removed by  
centrifugation at 14,000 g for 20 min at 4°C and supernatants stored at -20°C. One  
hundred µg of protein per lane was resolved by 7.5% SDS-polyacrylamide gel  
electrophoresis, transferred to nitrocellulose membranes (Amersham-Pharmacia  
Biotech, Chalfont St Giles, UK) and probed with anti-PR antibodies 1:50 (clone 1A6) or  
100 1:400 (C-20) in 3% Blotto. Peroxidase-conjugated secondary antibody (Amersham) was  
used at a 1:2000 dilution and the reaction visualised using ECL detection kits. Negative  
controls were performed with blots not exposed to primary antibody and by incubation  
of antibodies with a 7-fold excess of immunising peptide or extracts produced from  
choriocarcinoma (BeWo) cells transiently transfected with human PR-A (22) or PR-C  
105 (9) expression plasmids.

## Results

To investigate the PR isoform repertoire of the progesterone-dependent human fetal-maternal interface, immunohistochemical analysis using the commercial PR antibody clones 1A6 and C-20 generated towards the ligand- and DNA-binding domains, respectively, of the full length PR-B isoform were used (Fig1). The data revealed the presence of cytoplasmic staining in the amnion epithelial cell, the chorionic trophoblast and the maternal decidual cell. Nuclear staining with these antibodies was only found in decidual cells and positive control tissues such as breast cancer, endometrium and myometrium (positive control data not shown). No nuclear staining was detected in the amnion epithelium and chorionic cytotrophoblast (Fig 1). The connective tissue cells of the reticular and chorionic layers of the fetal membrane were devoid of any immunoreactivity (Fig1a). In the placenta, the syncytiotrophoblast also demonstrated cytoplasmic staining with this antibody and no nuclear staining (Fig 1c). However, cytoplasmic and nuclear immunoreactivity was observed in decidual cells attached to the basal plate (Fig 1c).

To confirm the identity of the cytoplasmic isoform found in these cell types, immunostaining with PR-B- and PR-A-specific antibodies revealed nuclear staining in the decidual cell, but no other staining elsewhere in the fetal membrane (Fig 1). Similarly, nuclear staining was confined only to the decidual cells of the basal plate attached to the placenta (Fig 1e). Nuclear staining in classical progesterone target tissues (breast cancer, endometrial, and myometrial cells) confirmed the PR-B and PR-A specificity of the antibodies (data not shown). The conclusion from these studies was that the cytoplasmic isoform found within fetal membranes, decidua and the syncytiotrophoblast of the placenta was neither the PR-B nor the PR-A isoform and suggestive of one of the other PR isoforms. These data were inconsistent with previous observations that the amnion epithelial cell contained both PR-B and PR-A isoforms

and that during labour there is a loss of the PR-B isoform in favour of the PR-A isoform (17), but is consistent with immunohistochemical studies of Goldman et al. (19).

To find the identity of the cytoplasmic progesterone receptor isoform, RNA  
135 extracts prepared from term fetal membrane and placenta samples were subjected to  
RT-PCR with several isoform-specific primer sets (Table 1) and revealed the presence  
of a PR transcript in all samples by RT-PCR (Fig 2a & 2b; top panels). Analysis with  
PR-B-, PR-A/PR-B- and PR-S-specific primer sets showed that fetal membranes with  
decidua attached consisted mainly of PR-A transcripts with some transcripts for PR-B,  
140 but no PR-S transcripts (Fig 2). Fetal membranes enriched for amniochorion revealed  
the presence of a transcript for PR that was not PR-B, not PR-A or PR-S leading to  
conclusion that the cytoplasmic PR isoform present was either PR-C or PR-M  
previously observed in human breast cancer cells and isolated from human aortic  
endothelial cells, respectively (6, 9, 23). The placenta similarly contained a transcript  
145 for PR that was either PR-C, or PR-M (Fig 2b). Samples enriched for decidua revealed  
the presence of all PR isoforms including PR-S (Fig 2b).

When protein extracts from partially purified fetal membranes, placenta,  
trophoblasts and decidua were compared with those from T47D breast cancer cells,  
using western blotting techniques with the PR antibodies that detected the cytoplasmic  
150 isoform in the immunohistochemistry studies (Fig 1; Table 2), several PR  
immunoreactive proteins were observed in T47D breast cancer cell extracts in line with  
previous studies (8, 19, 21), whereas the tissues at the fetal maternal interface revealed a  
major PR isoform with a relative molecular mass of ~60 kDa (Fig 3). This isoform was  
confirmed as the PR-C isoform, as previously reported in human breast cancer cells (5,  
155 23), human fetal membranes (19) and the guinea pig cervix (21), with recombinant  
protein and pre-adsorption studies (Fig 3). Other progesterone target tissues, such as

endometrium (data not shown) and myometrium possessed the 116 kDa PR-B, 94 kDa PR-A and 60 kDa PR-C isoforms but only with the C-20 antibody (Fig 3).

## Discussion

160 The role of progesterone receptors in human gestation and parturition is to maintain pregnancy. In most mammals, the stability of the relationship in the fetal-maternal interface is disturbed by the fall of progesterone production by the placenta, with the concomitant softening of the cervix, rupture of the fetal membranes and initiation of highly synchronised high pressure contractions of the myometrium that characterises  
165 labour (24). In the human, similar events occur in the same coordinated manner except there is no loss of systemic progesterone (24). A hypothesis presented (17), that a 'switch' in PR isoforms from the more transcriptionally dominant PR-B to the less active PR-A isoform must therefore occur in these tissues, has been convincingly supported by evidence in the myometrium (25, 26), and in the cervix (27, 28) and less  
170 convincingly in the amnion and maternal decidua (19).

In the present study we have shown that the fetal membranes (amnion epithelial cell and chorionic cytotrophoblast) contain mainly high levels of PR-C (Fig 1; Table 2), whereas the placental cytotrophoblast is devoid of PR and the placental syncytiotrophoblast contains both PR-C and PR-A (Table 2). By contrast, the maternal  
175 decidua contains not only PR-A, as its major isoform, but also PR-B, PR-S and the cytoplasmic PR-C. These data differ from those recently reported (19) where PR-B and PR-A isoforms were observed in amnion nuclear extracts yet, paradoxically, the antibody used failed to show immunoreactive PR by immunohistochemistry and theoretically, should not detect the PR-C, PR-S, or PR-M isoforms in western blotting,  
180 as the epitope for this antibody is found only at the N-terminal region of the PR-A isoform (29). In the present study, no PR-B or PR-A isoforms were detected in the

amnio or chorion and the hypothesis that progesterone receptor switching occurs within these tissues at term or in labour (17) is not supported. Although, we cannot rule out the presence of a small amount of PR-B or PR-A isoforms being present in the nuclei of amnio epithelial cells, despite a lack of expression of these isoforms at the mRNA level (Fig 2), the present study indicates that PR-B and PR-A are of little importance in relation to PR-C in fetal membranes. Additionally, the PR immunoreactivity in the present study that we consider represents the C isoform, was confined to the cytoplasm of the amniotic epithelium and the chorionic cytotrophoblast, and was the major isoform present, whereas no cytoplasmic immunoreactivity was detected in the aforementioned study (19). These data are qualitatively similar to that obtained in the baboon (30), where PR was not observed in the amnio or chorion, but strong nuclear and weak cytoplasmic staining was observed in the decidua with the JZB39 rat anti-PR monoclonal antibody. Observations obtained with JZB39 (31), which has been shown to only detect the PR-C isoform in myometrium and not T47D extracts by immunoblotting, were dependent upon protein concentrations (31). These data suggest that protein loading in immunoblotting is critical for good PR-C detection. Indeed, PR-C was weakly represented on western blotting in a previous study (19), whereas our data show intense PR-C expression (Fig 3) for this exact reason.

Alternatively, these discrepant observations may be related to the antibodies used, the use of nuclear extracts compared to whole cell extracts or the presence of contaminating cytoplasm/tissues in the previous study (19).

In agreement with our studies, Goldman et al. (19) showed that PR-A is expressed in the nucleus of the decidua but absent in the amnio by immunohistochemistry, but paradoxically, found all isoforms present by western blotting. By contrast, our western blot studies indicate the major PR isoform in the fetal membranes (amnio and chorion) is C and that it is localised within the cytoplasm, without immunoreactivity within the nuclei of the same cells and, therefore, we consider that the relevance of the changes in

levels of the PR isoforms detected within the amniotic nuclear extracts of labour (19),  
210 should be considered with caution.

Using antibodies that detect all known PR isoforms, we demonstrated that the major isoform in the amnion, chorion and syncytiotrophoblast is the 60kDa PR-C isoform (Fig 3). The absence of PR-B and PR-A transcripts in these tissues (Fig 2) confirmed these findings. By contrast, the major isoform in the term decidua is PR-A,  
215 but PR-B, PR-S and PR-C isoforms are also expressed. In light of the study by Goldman et al., these data need further clarification, but suggest that PR-C is an important molecule in the fetal membranes, decidua and syncytiotrophoblast. These data also suggest that a re-evaluation of the roles of PR in the fetal-maternal interface is needed and the idea that PR-A simply acts as a dominant negative regulator of PR-B action is  
220 not applicable to these tissues (17).

The mechanism involved in the synthesis of the PR-C isoform is unknown, but limited evidence suggests that it may be produced using a third oestrogen dependent promoter with an AUG at amino acid 595 being the translational start site (5). The demonstration that PR-C isoforms in the human breast cancer cell do not arise from  
225 proteolysis of larger PR-isoforms (9) and that specific PR-C mRNA transcripts are present in human fetal membranes and placenta (this study), strongly suggests that the PR-C isoform is generated through promoter specific transcription. The function of PR-C is unclear, although it may act as modulator of PR-A and PR-B transcriptional activities in those cells that produce these isoforms (9). In the human amnion and  
230 chorion, dominant negative regulation of PR-B and PR-A transcriptional control seems unlikely in the absence of any measurable PR-A or PR-B isoforms and point towards PR-C specific functions. Since PR-C lacks a full DNA binding domain (9), but has a nuclear localisation signal and two dimerisation domains, the intriguing possibility that PR-C may associate with other transcriptional elements to modulate gene transcription

235 is raised. However, the cytoplasmic location and relatively high levels of this protein are suggestive of a localised function in the cytoplasm rather than a nuclear genomic function and that this localisation is important for sustained gestation. It now seems imperative that the regulation and role of the PR-C isoform is investigated to further our understanding of the role of progesterone in these tissues during human parturition.

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## Figure Legends

**Figure 1.** Identification of PR-positive cell types at the fetal-maternal interface. **(Panel a)** Low power image of fetal membranes showing the immunohistochemical staining patterns obtained with antibodies that detect all isoforms of PR (clone 1A6; 1:50) and C-20; 1:40), antibodies that detects only PR-B (San27, 1:150) and antibodies that detect only PR-A (clone 16; 1:200). An equivalent amount of mouse immunoglobulins (IgG) or antibody pre-adsorbed with immunising peptide, against which C-20 antibodies were generated, indicates specificity. Immunoreactivity is indicated with the black arrows in the amniotic (a), the star in the chorionic (c) and with red arrows in the decidual (d) layers of the fetal membranes. **(Panel b)** The presence of cytoplasmic PR and an absence of PR-B and PR-A isoforms are demonstrated in term amnion epithelial cells (**1, 4, 7, and 10** respectively), and chorionic cytotrophoblasts (**2, 5, 8, and 11** respectively). Both cytoplasmic and strong nuclear staining was observed in the decidua (**3, 6, 9 and 12**) indicating the presence of cytoplasmic and nuclear PR isoforms. **(Panel c)** High power images of PR immunostaining with clone 1A6 indicating immunoreactivity in the cytoplasm of the amnion epithelial cell (1) and the chorionic cytotrophoblast (2). Note the absence of staining in the blue nucleus of either cell type. **(Panel d)** A low power image of PR immunostaining of the term placenta with clone 1A6 antibodies indicating immunoreactivity in the syncytiotrophoblast layer, but absent elsewhere. **(Panel e)** High power images of PR immunostaining of term placenta indicating the presence of cytoplasmic PR in the syncytiotrophoblast but not nuclear PR-B and PR-A isoforms (**panel e; 1, 2, and 3**); whereas basal plate decidual cells contained both cytoplasmic and nuclear PR isoforms (**4, 5, and 6 arrowed**). These data suggest the cytoplasmic PR staining in amnion epithelial cells, cytotrophoblasts, syncytiotrophoblasts and decidual cells, is not PR-B or PR-A, but PR-S, PR-M or PR-C. Data are representative of six independent samples. Images were obtained at 100x

magnification (**panel a**), 1000x magnification (**panel b**), 4000x magnification (**panel c**), 200x magnification (**panel d**), and 400x magnification (**panel e**).

**Figure 2. (Panel a)** Evidence that the PR isoform in human fetal membranes and placenta at term is PR-C. RT-PCR products generated from fetal membrane samples that had decidua attached (**column A**) or fetal membranes that had the decidua scraped away (**column B**) or term placenta (**column C**). One  $\mu\text{g}$  of DNase-treated total RNA was reverse transcribed in the presence (+), or absence (-), of AMV-RT. Both samples were then amplified in a PCR with isoform-specific primers (see Figure 1; Table 1) that detected either all known PR isoforms; the PR-B isoform alone; a combination of PR-A and PR-B; or the PR-S isoform alone. GAPDH amplification was used a control for efficiency of mRNA manipulation and PCR amplification. Myometrium (**column D**) was used as a positive control for PR-B, PR-A and PR-C with T47D cell extract as a positive control for PR-S (**separate column**) and in all cases a minus RT sample was incorporated to rule out the presence of contaminating genomic DNA. Results are representative of four independent assessments. Data show the presence of PR in all 4 tissue samples and T47D cells. The PR-B isoform in the myometrium was approximately equal with PR-A in expression levels. The PR-A isoform was predominantly expressed in the fetal membrane that has decidua attached to it (**column A**) although a small amount of PR-B was still visible. There was a PR isoform in fetal membrane lacking decidua (**column B**), but it was not PR-B, not PR-A nor PR-S. PCR for the housekeeping gene GAPDH was used to control for input cDNA levels. (**Panel b**) RT-PCR gels showing the PR transcripts found in extracts of enriched amnion (**column A**), chorion (**column B**) and decidua (**column C**). Data was obtained from the +RT sample only and indicate that the amnion contains a PR transcript that is not PR-A or PR-B. Additionally, enriched decidua contains the PR-S isoform whereas the chorion (which is contaminated with decidua) also contains PR-S, PR-B and PR-A. The amnion

does not contain these transcripts, but does contain a PR isoform. The conclusion is that this isoform is PR-C or PR-M.

**Figure 3.** Confirmation that the PR isoform in human fetal membranes at term is primarily PR-C. **(Panel A)** Protein extracts from fetal membrane samples that had decidua attached (**lane 1**) and fetal membranes that had the decidua scraped away (**lane 2**) were compared with extracts from term amnion (**lane 3**), chorion (**lane 4**) and decidua (**lane 5**) that were obtained from crude tissue separation methods and from term placenta (**lane 6**), myometrium (**lane 7**) and breast cancer (T47D cell) extracts (**lane 8**). The antibody clones used were 1A6 (upper series) that detects all PR isoforms (see lane 8) and C-20 (lower series) that detects additional isoforms (see lane 8). The data indicate the presence of a major PR isoform of ~60 kDa in all the fetal membrane samples and that tissues ‘contaminated’ with decidua were devoid of significant levels of the PR-B, but contain small amounts of PR-A isoforms that are easily detected in myometrium and T47D cell controls. Placenta and term decidua also indicate the presence of PR-C as the major PR isoform although other PR-isoforms were observed. The 38 kDa PR-M isoform was only observed in the tissues contaminated with deciduas and T47D extracts with these antibodies. **(Panel B)** Confirmation that the antibodies detect the 60kDa PR-C isoform was obtained by immunoblotting an extract obtained from human choriocarcinoma (BeWo) cells transiently transfected with a human PR-C and probed with C-20 antibodies (-) or C-20 antibodies pre-adsorped with immunising peptide (+). **(Panel C)** Further confirmation of nature of the main PR isoform in the amnion was obtained by probing immunoblot extracts of T47D (control) and term amnion (Am) with the 1A6 antibody clone pre-absorbed with BeWo cell extracts (no transfection), or BeWo extracts transiently transfected with human PR-C (hPRc) or PR-A (hPRa) expression plasmids. These data indicate that only hPRc significantly inhibited the immunoreactivity of the ~60 kDa band suggesting that this protein is the PR-C isoform.

**Table 1. Designation and sequences of primers used in RT-PCR.**

Primer	Sequence	Combinations	Size	Detects
pB5'	CCTgAAgTTTCggCCATACCT	p15 & p35	284	B, A, C, M
pB3'	AgCAgTCCgTgTCCTTTCT	p33 & p36	396	B & A
pS	gAATTCaggAgAgTgggTgCTC	pS & p33	186	S
p15	AggAgTTTgTCAAgCTTCAA	pB5' & pB3'	197	B
p33	gAATTCATTTggAACgCCCCTgg			
p35	CTgCAgggACTggATAAATgTATTC			
p36	CTgCAggTCTACCCgCCCTATC			
gapdf	AGAACATCATCCCTgCCTC	gapdf & gapdr	347	GAPDH
gapdr	gCCAAATTCgTTgTCATACC			

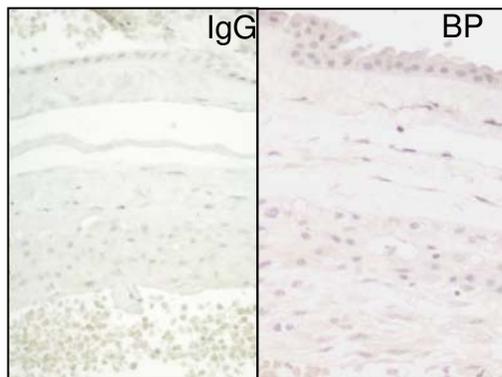
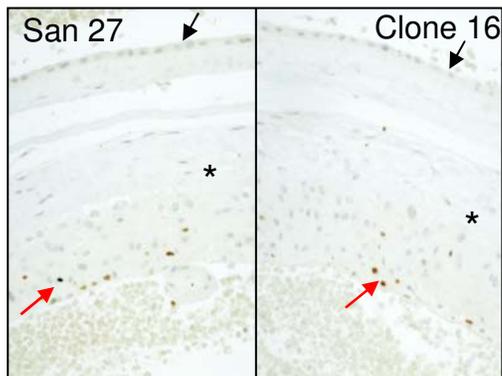
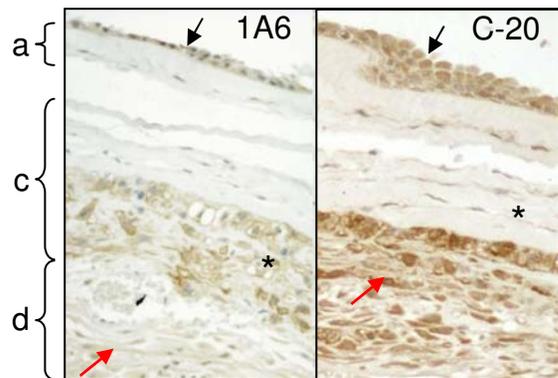
Primer combinations, expected amplicon size, reaction conditions and isoforms detected are shown in Fig 1 and are described in references 20 and 25.

**Table 2. Summary of PR isoform expression patterns**

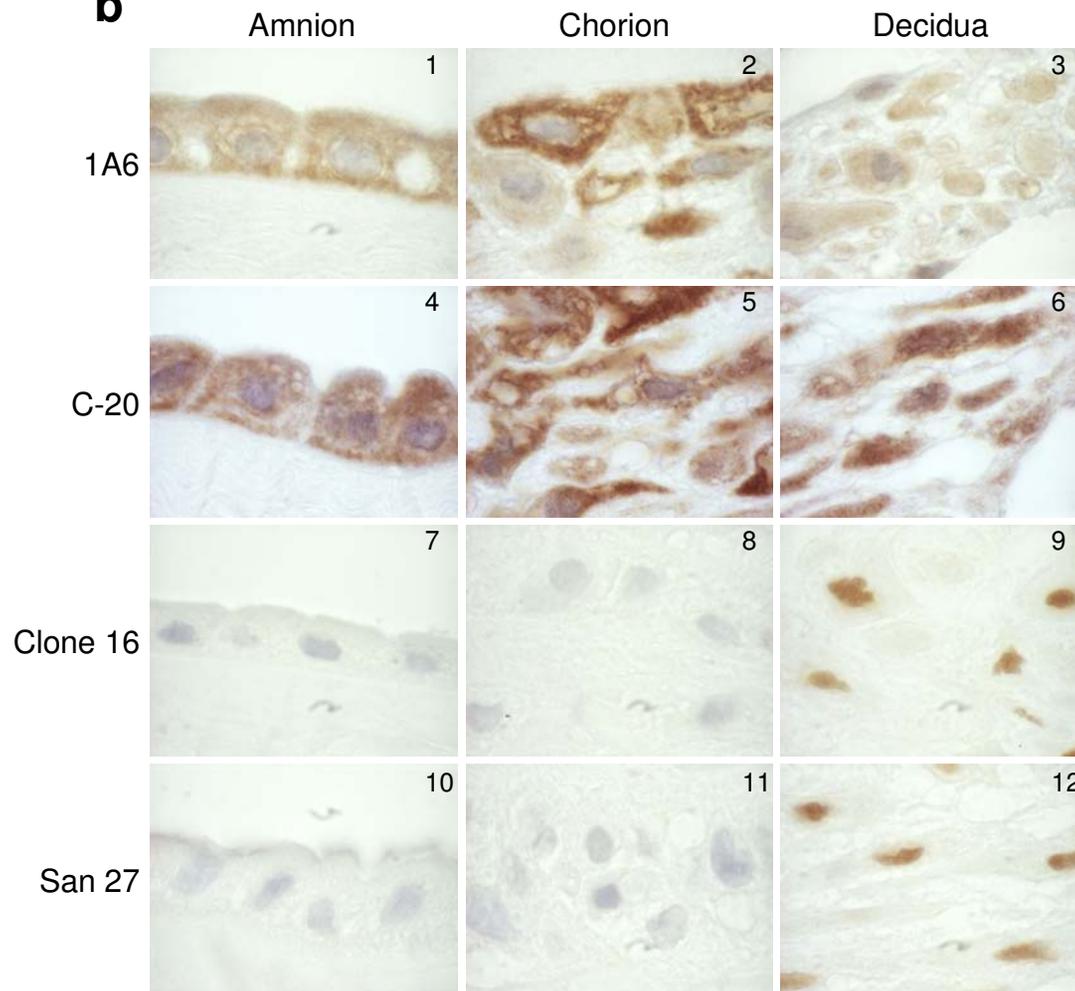
Tissue	PR-B <sup>1, 2, 3</sup>	PR-A <sup>1, 3</sup>	PR-C <sup>1, *</sup>	PR-S <sup>2</sup>	PR-M <sup>3</sup>
Amnion	-	-	+++	-	-
Chorionic cytotrophoblast	-	-	+++	-	N/D
Placental cytotrophoblast	-	-	-	-	N/D
Placental syncytiotrophoblast	-	-	+	-	N/D
Maternal decidua	++	+++	++	+	-

Presence determined by <sup>1</sup>Immunohistochemistry, <sup>2</sup>RT-PCR, or <sup>3</sup>Western blot. \* PR-C classified as cytoplasmic staining in immunohistochemistry; + weak presence, ++ moderate presence, or +++strong presence, N/D not determined.

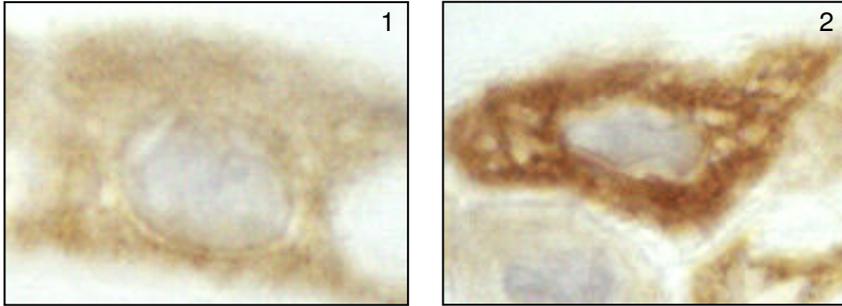
**a**



**b**



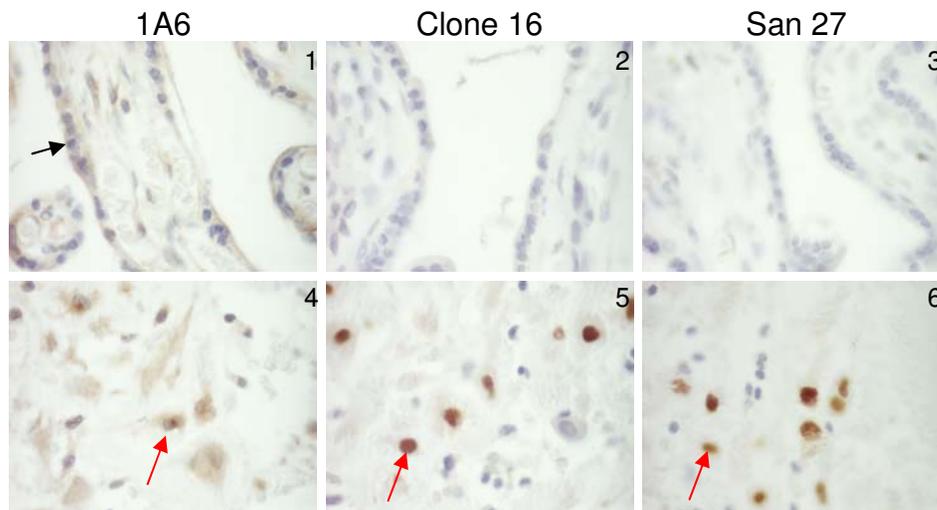
**c**

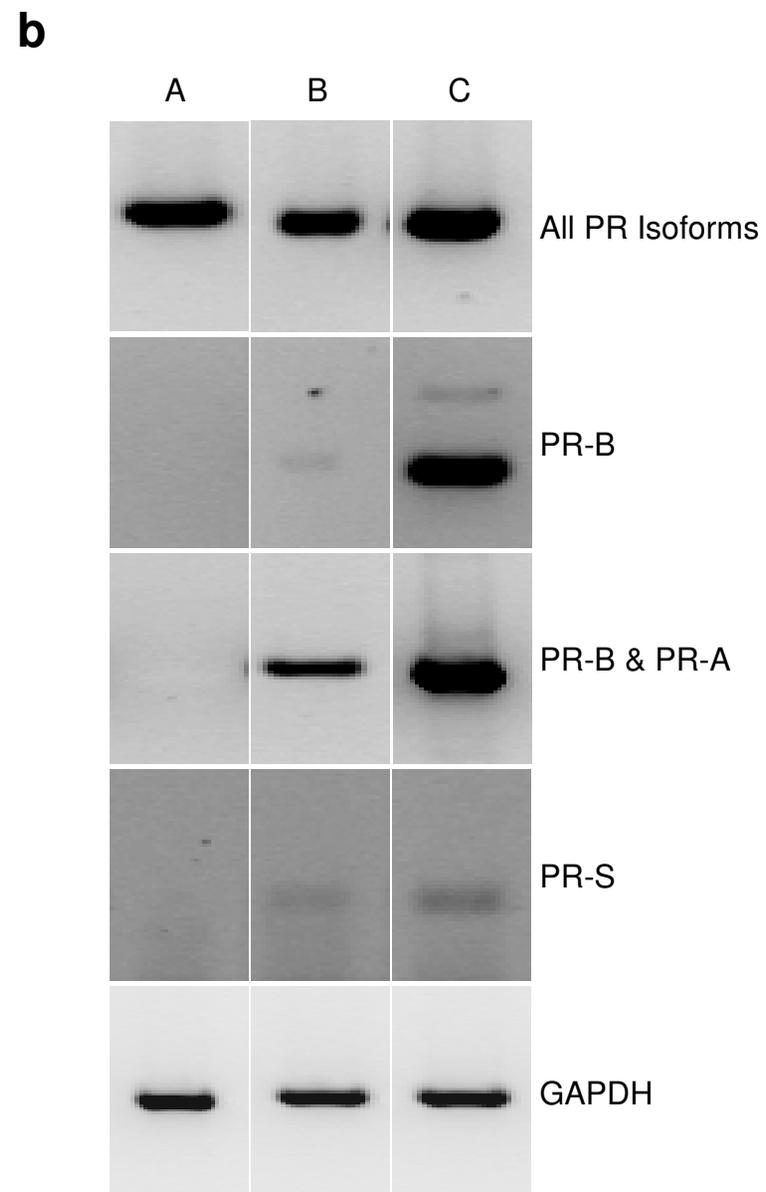
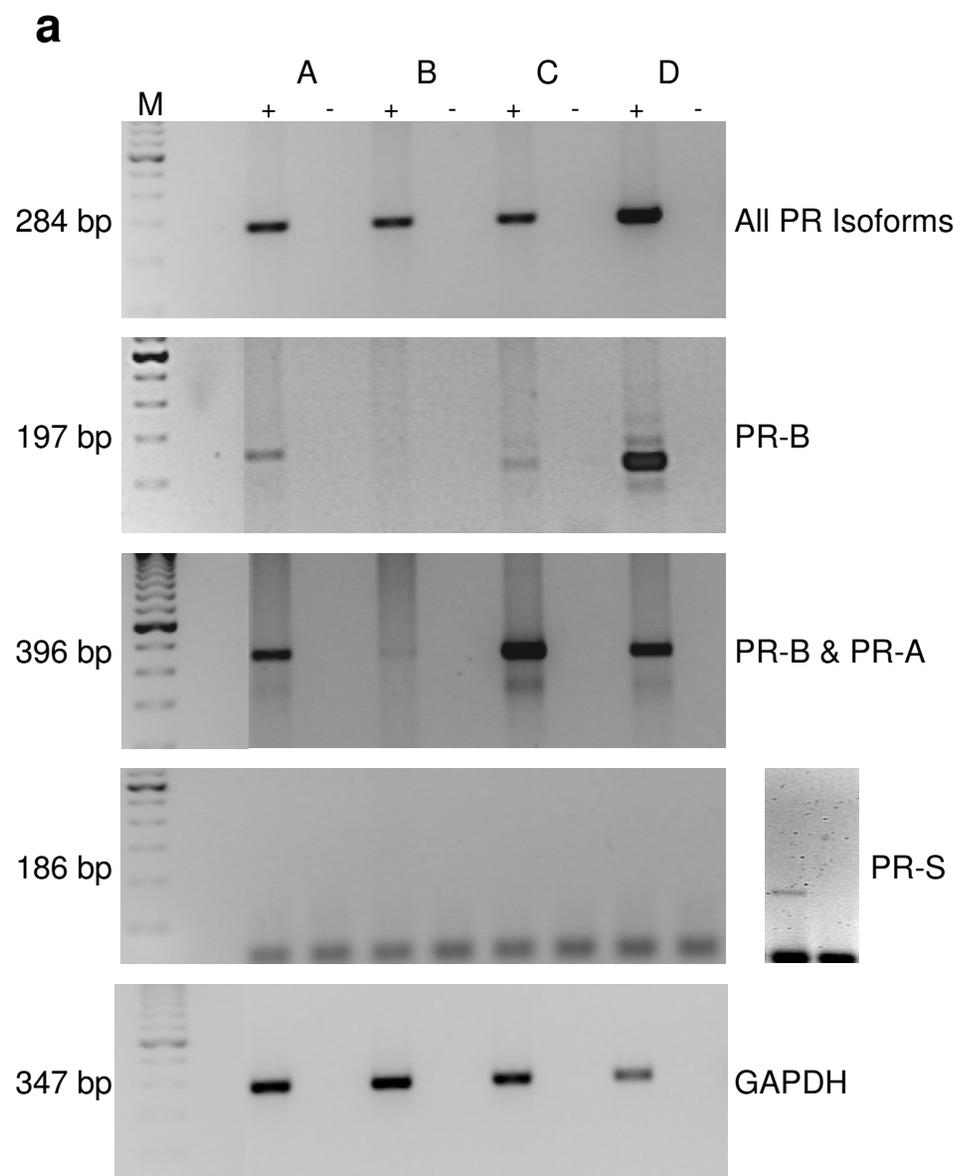


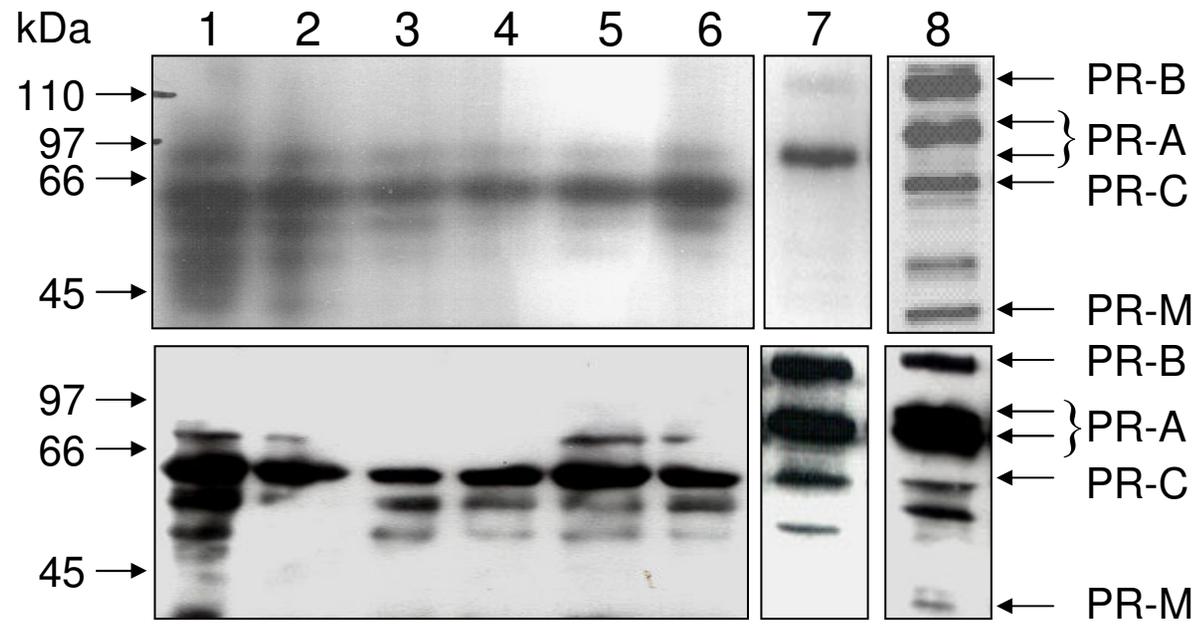
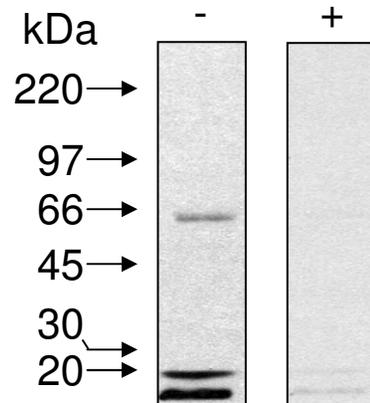
**d**



**e**





**A****B****C**