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The polypeptides and genes for ovine and bovine trophoblast protein-1

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Summary. Ovine and bovine trophoblast protein-1 (oTP-1 and bTP-1) have been strongly implicated as antiluteolytic agents and responsible for maternal recognition of pregnancy in sheep and cattle, respectively. Both are interferons (IFN) belonging to the IFN-α family, but their length (172 residues versus 166 for most IFN-α) places them in an unusual subclass (the IFN-α_{II}). The various isoforms of oTP-1 and bTP-1 produced by trophoblast tissue appear to arise in part from translation of multiple mRNAs which are themselves the products of distinct genes. These genes, like those for other IFN-α, are without introns. However, the genes for oTP-1 and bTP-1 form a distinct subgroup within the IFN-α_{II} on the basis of their overall primary sequences and the high conservation of the 3′-untranslated ends of their transcription units. The bTP-1 genes also differ from the bovine IFN-α_{II} in the organization of the promoter regions upstream from the transcription start site. Nevertheless, computer-aided analysis of the primary polypeptide sequences of oTP-1 and bTP-1 indicates that the molecules are likely to have approximately the same shapes and dimensions as all other IFN-α molecules. It remains to be determined whether they have unique biological properties which distinguish them from other IFN-α molecules.

Keywords: α-interferons; trophoblast; molecular cloning; sheep; cattle

Introduction

Ovine trophoblast protein-1 (oTP-1) and bovine trophoblast protein-1 (bTP-1) have been strongly implicated as mediators of maternal recognition of pregnancy in sheep and cattle (Bazer *et al.*, 1986). In particular, they appear to be major factors in preventing the regression of the corpus luteum in these two species. Ovine TP-1 is produced in major amounts by trophectoderm at around the time the conceptus begins to elongate from a sphere to a longer, thread-like form, i.e. about Day 13 (Hansen *et al.*, 1988; Farin *et al.*, 1989). On two-dimensional polyacrylamide gels oTP-1 is evident as 3 or 4 isoforms of M_r about 18 000 (Godkin *et al.*, 1982; Imakawa *et al.*, 1987). It is the main secretory product of conceptuses between Days 13 and 21 of pregnancy (Godkin *et al.*, 1982) and the major translation product of Day-16 conceptus RNA (Hansen *et al.*, 1985; Anthony *et al.*, 1988). It can extend the length of the oestrous cycle when infused into the uteri of non-pregnant ewes (Godkin *et al.*, 1984a; Vallet *et al.*, 1988) and appears to act as a paracrine hormone directed locally to the uterine endometrium where it influences protein synthesis (Godkin *et al.*, 1984b;

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Vallet *et al.*, 1987; Sharif *et al.*, 1989) and dampens the pulsatile output of the uterine luteolysin, prostaglandin F-2α (Fincher *et al.*, 1986).

The bovine homologue of oTP-1, bTP-1 also consists of several isoforms when analysed by two-dimensional electrophoresis, although it is a rather more basic protein than oTP-1 (Bartol *et al.*, 1985; Helmer *et al.*, 1987). In addition, however, there are at least two size classes of molecule (M_r 22 000 and 24 000). As reviewed in the next section, the isoelectric variants are probably the products of different genes, whereas the different size classes most probably arise from differential glycosylation. Major synthesis of bTP-1 is initiated around the time that a conceptus must be present in the uterus in order to prevent regression of the corpus luteum, i.e. around Day 15 (Farin *et al.*, 1990). As in the case of oTP-1, the induction of bTP-1 seems to be triggered at the time when the spherical blastocyst begins to elongate. However, the time of morphological transition seems less fixed in cattle than in sheep (unpublished results) so that in some conceptuses high concentrations of bTP-1 mRNA have been noted associated with elongating conceptuses as early as Day 12–13 (Farin *et al.*, 1990). In addition, bTP-1 synthesis is spread over a much longer period of conceptus development. Godkin *et al.* (1988), for example, have noted bTP-1 production as late as Day 36 of pregnancy, while oTP-1 synthesis is essentially completed by Day 23 (Godkin *et al.*, 1982; Farin *et al.*, 1989). Like oTP-1, bTP-1 seems to function as an antiluteolysin. Injection of total conceptus secretory proteins (Knickerbocker *et al.*, 1986a) or of purified bTP-1 (Helmer *et al.*, 1989) into the uterine lumina of non-pregnant recipient cows can extend luteal function significantly, and this effect is most probably due to an inhibition of pulsatile PGF-2α output by the endometrium (Knickerbocker *et al.*, 1986b). These various aspects of the biology of oTP-1 and bTP-1 are discussed in further detail by Flint *et al.* (1991), Salamonsen *et al.* (1991) and Bazer *et al.* (1991).

Molecular cloning of the mRNA for oTP-1 and bTP-1

Amino acid sequencing of the NH_2 terminal of oTP-1 (Imakawa *et al.*, 1987; Stewart *et al.*, 1987; Charpigny *et al.*, 1988) and the cloning of oTP-1 (Imakawa *et al.*, 1987) and bTP-1 (Imakawa *et al.*, 1989) cDNA revealed that both proteins were related to the alpha-interferon (IFN-α) family of proteins. More specifically, they appeared to belong to a subgroup of IFN-α whose members are 172 rather than 166 residues in length. This subgroup has been named IFN-α_{II} by Capon *et al.* (1985) or IFN-ω by Hauptmann & Swetly (1985) to distinguish it from the 166-residue type (usually referred to as IFN-α_I). A comparison of the nucleotide and inferred amino acid sequences of a bTP-1 and a bovine IFN-α_{II} cDNA is shown in Fig. 1. It should be noted that these cDNA molecules are very similar to each other and diverge most markedly in their 3′-untranslated termini.

The multiple isoforms of oTP-1 and bTP-1 which are evident during two-dimensional electrophoresis suggested that each protein may be translated from more than a single species of mRNA (Anthony *et al.*, 1988). Indeed, the presence of multiple transcripts immediately became evident in the initial cDNA cloning studies (Imakawa *et al.*, 1987, 1989; Stewart *et al.*, 1989). We have obtained the sequences of 7 cDNA clones for oTP-1 that appear close to full length. The differences in nucleotide sequence and inferred amino acid sequence within the 195 codon open reading frame are summarized in Fig. 2. There appear to be two major subtypes (oTP-1A and oTP-1B). The upper 5 clones are of the B type (201, p3, p5, p7 and p8) and are very similar to each other. A total of 9 base differences accounting for 7 amino acid substitutions are evident among the 5 clones. Because some of these substitutions would result in charge alterations, e.g. gln for glu at position 5, asp for gly at position 101 and lys for glu at 107, the pI of the resulting proteins would be expected to differ.

The second group of cDNA (Group A) represented by clones p6 and p12 were very similar to each other but differed quite extensively from the B group in both nucleotide and inferred amino acid sequence. The most interesting feature of Group A was the substitution of an A for G in codon

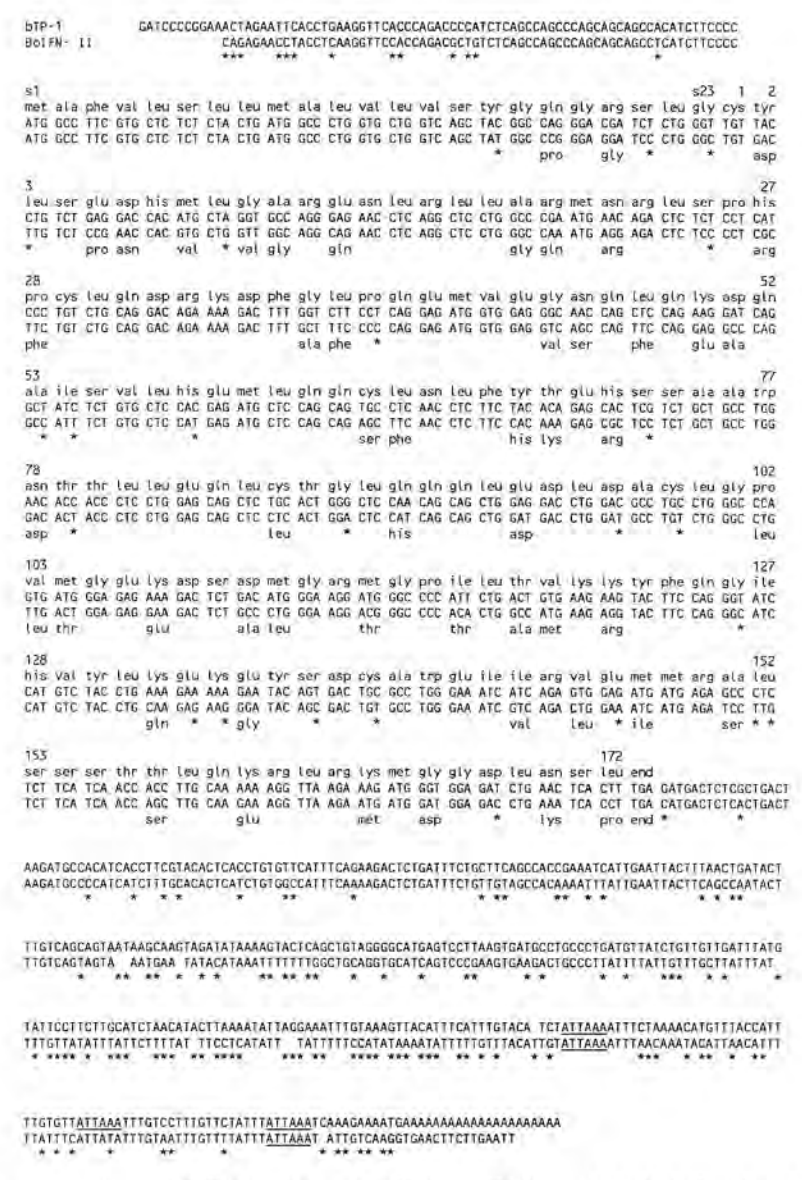

Fig. 1. A comparison of bTP-1 and bovine IFN-α_{II} cDNA and their deduced polypeptide sequences. The deduced amino acid sequence of bTP-1 is given above the nucleotide sequence. Amino acid residues differing in bovine IFN-α_{II} are indicated below the nucleotide sequence. Asterisks mark differences between the nucleotide sequences that do not result in a change in amino acid residue. Potential polyadenylation signals are underlined. Asn^{78} constitutes a potential site for N-glycosylation in bTP-1.

101 to provide an asn instead of an asp at residue 78 of the mature protein. This substitution creates a potential site for N-glycosylation on oTP-1 which is identical to the one thought to carry carbohydrate on bTP-1. However, there is no evidence that any of the forms of oTP-1 so far identified are glycosylated (Anthony *et al.*, 1988). By contrast, all of the cDNA for bTP-1 that we have so far sequenced code for asn at position 78, and all of the isoforms of bTP-1 appear to be glycoproteins (Anthony *et al.*, 1988; Helmer *et al.*, 1988). Interestingly, the 3′-ends of all 7 cDNA for oTP-1

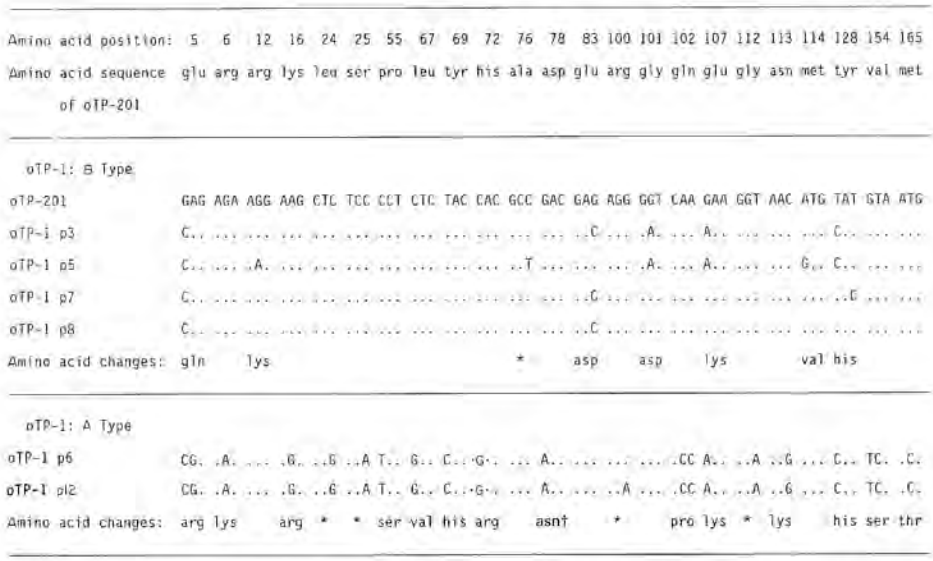

Fig. 2. A summary of nucleotide differences within the coding region of 7 different full-length cDNA for oTP-1 that have been cloned and sequenced in this laboratory. Two types of oTP-1 cDNA have been identified arbitrarily on the basis of degree of sequence identity and the presence of a potential codon for asn[78]. Sequences for 5 cDNA clones representing the B-type (201, p3, p5, p7, p8) are shown in the upper part of the diagram. Base changes and any resulting alteration in amino acid sequence are shown for each of the codons within the open reading frame of the cDNA. The single base change which should give no alteration in amino acid sequence in clone p5 is designated with an asterisk: 9 base differences giving rise to changes in 7 amino acids were noted among the 5 clones.

The A-type of cDNA (clones p6 and p12) is shown in the bottom part of the diagram. These two cDNA differed from clone 201 at 19 or more base positions within the open reading frame. The G→A substitution in the first position of codon 78 would result in the substitution of an asn (†) for asp and creates a potential site for N-glycosylation. These clones would also be expected to produce proteins of more basic pI than the A-type. Silent changes are again marked with an asterisk.

showed very little variability. A total of only 8 base changes were noted among all of the 7 clones (data not shown).

These data provide a basis to explain the multiple isoforms of oTP-1 and bTP-1. However, because the cDNA libraries were prepared from mRNA derived from several embryos, it is presently impossible to determine how many of the different cDNA shown in Fig. 2 and reported in earlier publications (Imakawa *et al.*, 1987, 1989; Stewart *et al.*, 1989) represent transcripts of different genes or allelic forms of the same gene.

Primary and deduced secondary structures of the trophoblast interferons

The trophoblast proteins oTP-1 and bTP-1 clearly resemble the IFN-α_{II} more than the more familiar IFN-α_{I} both in terms of the lengths of their polypeptides and in amino acid sequence (Imakawa *et al.*, 1989). The similarity to the predicted sequence of a bovine IFN-α_{II} is 70% compared to a 47–54% identity for cattle, human, rodent and pig IFN-α_{I}. However, oTP-1 and bTP-1 clearly resemble each other (~80% identity) more than they do the bovine IFN-α_{II}. Nevertheless, they possess several regions of sequence conserved in all IFN-α molecules so far characterized, including the 4 cysteines which participate in intrachain disulphide bonds, and a stretch of peptide near the carboxyl terminus. Moreover, the sequence similarity of oTP-1 and bTP-1 to IFN-α occurs

throughout the lengths of their polypeptide chains and is not confined to specific regions. It should also be recognized that the IFN-α_I molecules are themselves a highly divergent group of proteins (Gillespie *et al.*, 1984; DeMaeyer & DeMaeyer-Guignard, 1988). In general, they rarely show more than 60% sequence identity even between closely related species. Indeed, this diversity in sequence and multiplicity of the IFN-α_I genes themselves has raised questions as to whether their products might have subtly different functions.

A prediction of secondary structures by the procedure of Hopp & Woods (1981) has shown that oTP-1 and bTP-1 are likely to possess a considerable amount of alpha helical structure (Roberts *et al.*, 1990) as do other IFN (Zoon & Wetzel, 1984). Hydrophilicity profiles for an oTP-1, a bTP-1, a bovine IFN-α_I and a bovine IFN-α_{II} are shown in Fig. 3 for the 166 residues over which these 4 proteins overlap in sequence. The profiles for each IFN are very similar, and the data suggest that the overall tertiary structures of these molecules are likely to be conserved, i.e. the molecules will have approximately the same shapes and dimensions even though minor details of their architecture may differ.

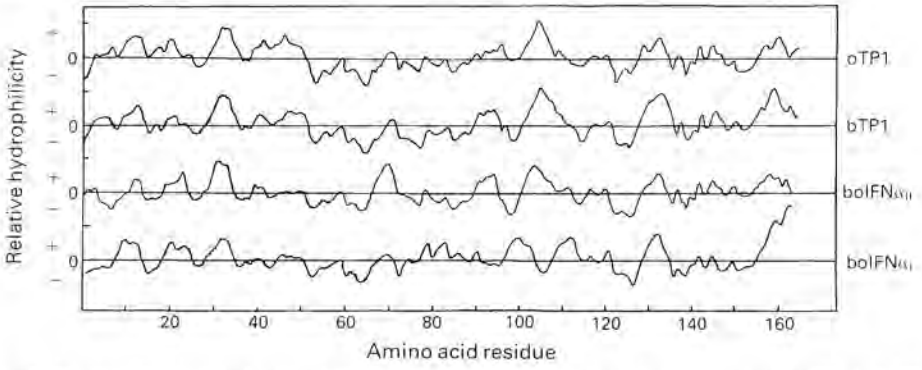

Fig. 3. Predicted relative hydrophilicity profiles for the inferred structures of oTP-1, bTP-1, bovine IFN-α_I and bovine IFN-α_{II} by using the algorithm of Hopp & Woods (1981). Sequences are compared over the first 166 residues only by using Microgenie© software from Beckman Inc. Regions plotted above the horizontal lines are relatively hydrophilic, those below the lines are hydrophobic. The figure is reprinted from Roberts *et al.* (1990).

Do trophoblast IFN molecules constitute a distinct IFN subgroup?

Table 1 summarizes nucleotide sequence differences between the transcripts for oTP-1, bTP-1, a bovine IFN-α_{II} and bovine IFN-α_I1 in the 5′- and 3′-untranslated termini and within the protein coding region. These data illustrate two main points. First, it is clear that the oTP-1 and bTP-1 transcripts resemble each other more than they do the apparently related bovine IFN-α_{II} transcript. Second, the region of greatest similarity between the oTP-1 and bTP-1 transcripts lies within the 3′-untranslated region of the molecules. This high degree of conservation in the 3′-ends persists when all the cDNA variants for oTP-1 and bTP-1 are considered (data not shown). By contrast, the same 3′-region provides the greatest degree of dissimilarity with the bovine IFN-α_{II} transcript (see also Fig. 1). Although it is unclear why the 3′-ends of the trophoblast IFN mRNA should be so highly conserved, it seems likely to have some functional significance and suggested to us that these IFN proteins might belong to a distinct subgroup within the larger IFN-α_{II} family.

It is possible to exploit the use of 3′-specific cDNA probes for oTP-1 and bTP-1 to distinguish mRNA for the trophoblast IFN from those for other IFN by carefully controlling hybridization conditions during in-situ hybridization procedures (Farin *et al.*, 1989, 1990). By using such methodology the onset of oTP-1 and bTP-1 gene expression can be accurately pinpointed during

Table 1. Comparison of the base sequences within the 5′ and 3′ non-coding regions and within the coding regions of cDNA for bTP-1, oTP-1, a bovine IFN-α_{II} and a bovine IFN-α_{I}

IFN	5′-Non-coding (%)	Coding (%)	3′-Non-coding (%)	Predicted amino acid sequence identity (%)
bTP-1	100	100	100	100
oTP-1	76	90	92	80
bIFN-α_{II}	72	85	70	72
bIFN-α_{I}	53	63	53	50

The sequences compared were λbTP-509 (Imakawa *et al.*, 1989), oTP-1 clone 201 (see Fig. 2), and the bovine IFN-α_{I} and -α_{II} genomic clones described by Capon *et al.* (1985). The 5′-non-coding region represents the stretch of nucleotides 5′ to the ATG initiation codon. The 3′-non-coding region lies between the termination codon and the poly(A) tail. Sequence comparisons were only performed on aligned regions with gaps ignored. The last 18 nucleotides of bTP-1, oTP-1 and bIFN-α_{II} cDNA were compared with each other but were excluded in comparisons with α_{I} cDNA.

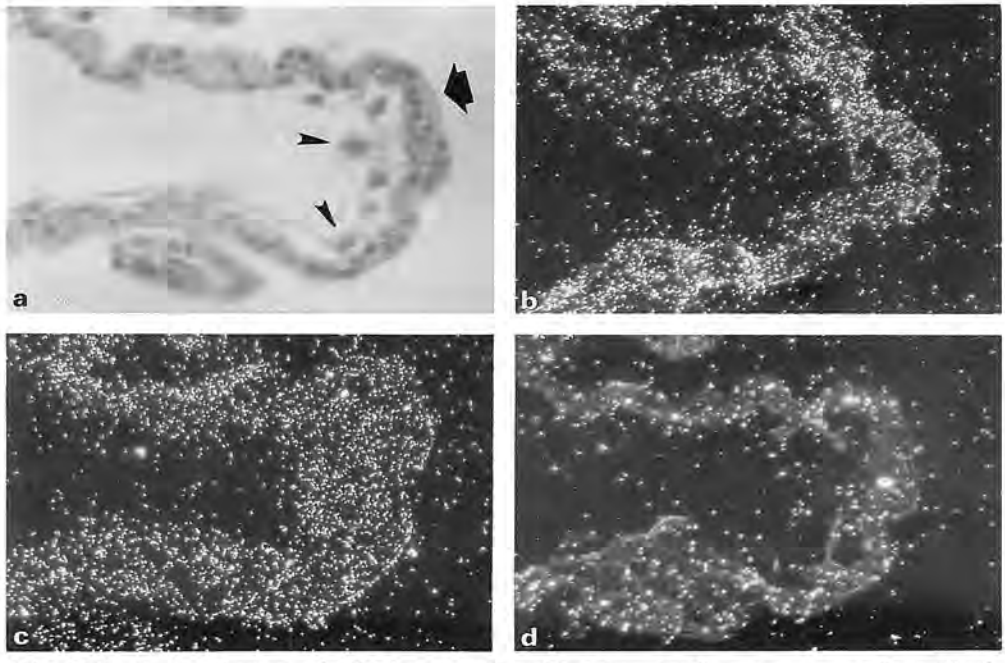

Fig. 4. In-situ localization of bTP-1 and actin mRNA in a bovine conceptus at Day 19 of pregnancy. (a) A bright-field micrograph (8 μm) stained with toluidine blue. Large arrow indicates trophectoderm cells; small arrows show underlying extraembryonic endoderm. (b) A dark-field micrograph on an adjacent section which had been hybridized to [^{35}S]oTP 266 cDNA. The latter has 92% identity with bTP-1 mRNA. Silver grains, indicative of a positive hybridization, appear white. Note that the positive signal is confined to trophectoderm. (c) A section adjacent to those in (a) and (b) which had been hybridized to a [^{35}S]γ-actin cDNA. Here a signal is also observed over the endoderm. (d) A control section which had been hybridized to ^{35}S-labelled vector cDNA. Note the low numbers of silver grains over the tissue. For details of the procedures, including the specific activities of the probes, see Farin *et al.* (1990).

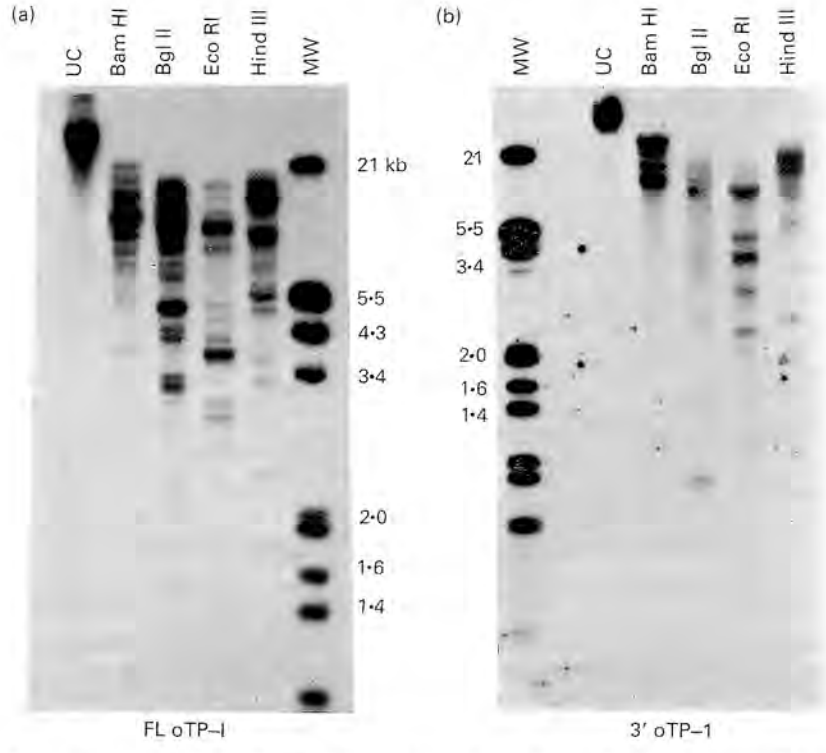

Fig. 5. Southern blot analysis of restriction endonuclease-digested ovine genomic DNA with a full-length ovine cDNA for oTP-1 (a) or a cDNA representing the 3′-untranslated end of the mRNA (b). Sheep genomic DNA was digested to completion with the indicated restriction endonucleases and separated by electrophoresis along with uncut (UC) DNA. (a) A Southern blot hybridized with a full-length (FL) oTP-1 cDNA probe which probably recognizes both oTP-1 and IFN-α_{II}, and related gene sequences. (b) Hybridization was with a 3′ oTP-1 probe (oTP-266; bp 650–912) which is specific for oTP-1. Densitometric scanning of the autoradiographs suggests that some of the darker hybridizing bands may represent restriction fragments containing multiple genes. Sizes of molecular weight markers are indicated in kb.

embryonic development. These mRNA are confined to the trophectoderm (Fig. 4) and begin to increase at around the time that the conceptus starts to elongate from a sphere to a longer thread-like form which in sheep is around Day 13 and in cattle around Day 15 (Farin *et al.*, 1989, 1990).

A similar approach has more recently been used to identify specific bTP-1 and bovine IFN-α_{II} genes. For example, we have screened a bovine genomic library with 3′-specific cDNA probes and isolated several bTP-1 and IFN-α_{II} clones from cross-hybridizing bacteriophage plaques. The identity of each DNA insert was further confirmed by means of the polymerase chain reaction in conjunction with specific oligonucleotide primers, and the product was subcloned and sequenced. The bTP-1 genes, like those of IFN-α_I and -α_{II}, were intronless. The protein coding region had between 95 and 100% sequence identities with known cDNA molecules for bTP-1. They possessed a TATA box at position -27 to -33 from the likely transcription start site and two putative viral response elements (VRE) (Fujita *et al.*, 1987; Raj *et al.*, 1989) 14 bp apart at base positions -69 to -74 and -88 to -93. The 5′-region of the bTP-1 gene was distinct in both overall sequence and in the placement of the VRE when compared with three IFN-α_{II} genes. These results indicate that the bTP-1 genes have diverged significantly from related IFN-α_{II} genes in their promoter regions.

Interestingly, however, they may still be potentially inducible by virus since they possess so-called virus response elements.

Specific 3′-end cDNA probes have also been used in Southern blotting experiments to determine the relative number of IFN-α_{II} and trophoblast IFN genes. Genomic DNA derived from leucocytes of individual cows or sheep was digested with a range of restriction endonucleases, Southern blotted and allowed to hybridize with ^{32}P-labelled cDNA probes representing oTP-1, bTP-1 or bovine IFN-α_{II}. Figure 5, for example, shows that when a full-length oTP-1 cDNA was used to probe restriction endonuclease-digested ovine genomic DNA, at least 15 bands greater than 1 kb in length were detected in some of the lanes. However, when a more specific probe representing the 3′-noncoding end of the cDNA (bases 650–912) was used, a maximum of only about five cross-hybridizing bands were evident (Fig. 4B). Essentially similar results have been obtained with bovine DNA. These data again emphasize that there are several genes for oTP-1 and bTP-1 and that these genes may be distinct from the IFN-α_{II} identified earlier by Capon *et al.* (1985).

Discussion

There have been several recent papers which have reviewed the evidence that the trophoblast IFN substances are mediators of maternal recognition of pregnancy in cattle and sheep (Bazer *et al.*, 1986; Roberts *et al.*, 1990). Although there seems little doubt that oTP-1 and bTP-1 function as antiluteolytic agents, it remains unresolved as to whether other compounds produced by the conceptuses are required to supplement the activity of oTP-1 and bTP-1 to cause an extension of the interoestrous interval for more than a few days. In addition, it is still not clear whether other IFN-α molecules can fully mimic the action of the trophoblast IFN on reproductive parameters (Plante *et al.*, 1988; Stewart *et al.*, 1989; Salamonsen *et al.*, 1991). This question will be more easily answered once recombinant bTP-1 and oTP-1 become available in workable quantities.

The experiments reviewed in this paper do strongly suggest, however, that bTP-1 and oTP-1, although 172 residues long, are distinct from other IFN-α_{II} proteins in a number of important respects. The very different organizations of IFN-α_{II} and bTP-1 genes in the 5′-promoter regions indicate, for example, that the two types of IFN might be under distinct types of transcriptional control. Indeed, in preliminary studies we have shown that bTP-1 is very poorly expressed in Sendai virus-induced leucocytes and IFN-α_{I} and -α_{II} transcripts are rare in Day 19 trophoblast tissue (unpublished results). Nevertheless, the fact that the bTP-1 gene does carry putative viral response elements and that oTP-1 is inducible by synthetic double-stranded RNA in Day-11 sheep conceptuses (Roberts *et al.*, 1990) is consistent with the hypothesis that genes for the trophoblast IFN are virally responsive, even if viral induction does not constitute the normal basis for oTP-1 and bTP-1 expression during pregnancy. It remains to be determined whether the uterine secretions of the pregnant ewe or cow contain factors responsible for the massive increase in transcription of the oTP-1 and bTP-1 genes around the time of maternal recognition of pregnancy.

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References

Anthony, R.V., Helmer, S.D., Sharif, S.F., Roberts, R.M., Hansen, P.J., Thatcher, W.W. & Bazer, F.W. (1988) Synthesis and processing of ovine trophoblast protein-1 and bovine trophoblast protein-1, conceptus secretory proteins involved in the maternal recognition of pregnancy. *Endocrinology* **123,** 1274–1280.

Bartol, F.F., Roberts, R.M., Bazer, F.W., Lewis, G.S., Godkin, J.D. & Thatcher, W.W. (1985) Characterization of proteins produced *in vitro* by peri-attachment bovine conceptuses. *Biol. Reprod.* **32,** 681–694.

Bazer, F.W., Vallet, J.L., Roberts, R.M., Sharp, D.C. & Thatcher, W.W. (1986) Role of conceptus secretory products in establishment of pregnancy. *J. Reprod. Fert.* **76,** 841–850.

Bazer, F.W., Thatcher, W.W., Hansen, P.J., Mirando, M.A., Ott, T.L. & Plante, C. (1991) Physiological

mechanisms of pregnancy recognition in ruminants. *J. Reprod. Fert., Suppl.* **43,** 39–47.

Capon, D.J., Shepard, H.M. & Goeddel, D.V. (1985) Two distinct families of human and bovine interferon-α genes are coordinately expressed and encode functional polypeptides. *Molec. cell. Biol.* **5,** 768–779.

Charpigny, G., Reinaud, P., Huet, J.-C., Guillomot, M., Charlier, M., Pernollet, J.-C. & Martal, J. (1988) High homology between a trophoblast protein (trophoblastin) isolated from ovine embryo and α-interferons. *FEBS Lett.* **228,** 12–16.

DeMaeyer, E. & DeMaeyer-Guignard, J. (1988) *Interferons and Other Regulatory Molecules.* John Wiley & Sons, New York.

Farin, C.E., Imakawa, K. & Roberts, R.M. (1989) *In situ* localization of mRNA for the interferons, ovine trophoblast protein-1, during early embryonic development of the sheep. *Molec. Endocrinol.* **3,** 1099–1107.

Farin, C.E., Imakawa, K., Hansen, T.R., McDonnell, J.J., Murphy, C.N., Farin, P.W. & Roberts, R.M. (1990) Expression of trophoblastic interferon genes in sheep and cattle. *Biol. Reprod.* **43,** 210–218.

Fincher, K.B., Bazer, F.W., Hansen, P.J., Thatcher, W.W. & Roberts, R.M. (1986) Ovine conceptus secretory proteins suppress induction of uterine prostaglandin F-2α release by oestradiol and oxytocin. *J. Reprod. Fert.* **76,** 425–433.

Flint, A.P.F., Parkinson, T.J., Stewart, H.J., Vallet, J.L. & Lamming, G.E. (1991) Molecular biology of trophoblast interferons and studies of their effects *in vivo*. *J. Reprod. Fert., Suppl.* **43,** 13–25.

Fujita, T., Shibuya, H., Hotta, H., Yamanishi, K. & Taniguchi, T. (1987) Interferon-β gene regulation: tandemly repeated sequences of a synthetic 6 bp oligomer function as a virus-inducible enhancer. *Cell* **49,** 357–367.

Gillespie, D., Pequignot, E. & Carter, W.E. (1984) Evolution of interferon genes. In *Handbook of Experimental Pharmacology*, vol. 71, pp. 45–63. Eds P. E. Carne & W. A. Carter. Springer-Verlag, New York.

Godkin, J.D., Bazer, F.W., Moffatt, J., Sessions, F. & Roberts, R.M. (1982) Purification and properties of a major, low molecular weight protein released by the trophoblast of sheep blastocysts at Day 13–21. *J. Reprod. Fert.* **65,** 141–150.

Godkin, J.D., Bazer, F.W. & Roberts, R.M. (1984a) Ovine trophoblast protein 1, an early secreted blastocyst protein, binds specifically to uterine endometrium and affects protein synthesis. *Endocrinology* **114,** 120–130.

Godkin, J.D., Bazer, F.W., Thatcher, W.W. & Roberts, R.M. (1984b) Proteins released by cultured Day 15–16 conceptuses prolong luteal maintenance when introduced into the uterine lumen of cyclic ewes. *J. Reprod. Fert.* **71,** 57–64.

Godkin, J.D., Lifsey, B.J., Fujii, D.K. & Baumbach, G.A. (1988) Bovine trophoblast protein-1: purification, antibody production, uterine cell interaction and antiviral activity. *Biol. Reprod.* **38** (Suppl. 1), 79, abstr.

Hansen, P.J., Anthony, R.V., Bazer, F.W., Baumbach, G.A. & Roberts, R.M. (1985) *In vitro* synthesis and secretion of ovine trophoblast protein-1 during the period of maternal recognition of pregnancy. *Endocrinology* **117,** 1424–1430.

Hansen, T.R., Imakawa, K., Polites, H.G., Marotti, K.R., Anthony, R.V. & Roberts, R.M. (1988) Interferon RNA of embryonic origin is expressed transiently during early pregnancy in the ewe. *J. biol. Chem.* **263,** 12801–12804.

Hauptmann, R. & Swetly, P. (1985) A novel class of human type I interferons. *Nucl. Acids Res.* **13,** 4739–4749.

Helmer, S.D., Hansen, P.J., Anthony, R.V., Thatcher, W.W., Bazer, F.W. & Roberts, R.M. (1987) Identification of bovine trophoblast protein-1, a secretory protein immunologically related to ovine trophoblast protein-1. *J. Reprod. Fert.* **79,** 83–91.

Helmer, S.D., Hansen, P.J. & Thatcher, W.W. (1988) Differential glycosylation of the components of the bovine trophoblast protein-1 complex. *Molec. cell. Endocrinol.* **58,** 103–107.

Helmer, S.D., Hansen, P.J., Thatcher, W.W., Johnson, J.W. & Bazer, F.W. (1989) Intrauterine infusion of highly enriched bovine trophoblast protein-1 complex exerts an antiluteolytic effect to extend corpus luteum lifespan in cyclic cattle. *J. Reprod. Fert.* **87,** 89–101.

Hopp, T.P. & Woods, K.R. (1981) Prediction of protein antigenic determinants from amino acid sequences. *Proc. natn. Acad. Sci. USA* **78,** 3824–3828.

Imakawa, K., Anthony, R.V., Kazemi, M., Marotti, K.R., Polites, H.G. & Roberts, R.M. (1987) Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm. *Nature, Lond.* **330,** 377–379.

Imakawa, K., Hansen, T.R., Malathy, P-V., Anthony, R.V., Polites, H.G., Marotti, K.R. & Roberts, R.M. (1989) Molecular cloning and characterization of complementary deoxyribonucleic acids corresponding to bovine trophoblast protein-1: A comparison with ovine trophoblast protein-1 and bovine interferon-α_{II}. *Molec. Endocrinol.* **3,** 127–139.

Knickerbocker, J.J., Thatcher, W.W., Bazer, F.W., Drost, M., Barron, D.H., Fincher, K.B. & Roberts, R.M. (1986a) Proteins secreted by Day 16 to 18 bovine conceptuses extend corpus luteum function in cows. *J. Reprod. Fert.* **77,** 381–391.

Knickerbocker, J.J., Thatcher, W.W., Bazer, F.W., Barron, D.H. & Roberts, R.M. (1986b) Inhibition of uterine prostaglandin $F_2\alpha$ production by bovine conceptus secretory proteins. *Prostaglandins* **31,** 777–793.

Plante, C., Hansen, P.J. & Thatcher, W.W. (1988) Prolongation of luteal lifespan in cows by intrauterine infusion of recombinant bovine alpha-interferon. *J. Endocr.* **122,** 2342–2344.

Raj, N.B.K., Israeli, R., Kellum, M. & Pitha, P.M. (1989) Upstream regulatory elements of murine α_4-interferon gene confer inducibility and cell type-restricted expression. *J. biol. Chem.* **264,** 11149–11157.

Roberts, R.M., Farin, C.E. & Cross, J.C. (1990) Trophoblast proteins and maternal recognition of pregnancy. *Oxford Rev. Reprod. Biol.* In Press.

Salamonsen, L.A., Cherny, R.A. & Findlay, J.K. (1991) In-vitro studies of the effects of interferons on endometrial metabolism. *J. Reprod. Fert., Suppl.* **43,** 27–38.

Sharif, S.F., Francis, H., Keisler, D.H. & Roberts, R.M. (1989) Correlation between the release of ovine trophoblast protein-1 by the conceptus and the production of polypeptides by the maternal endometrium of ewes. *J. Reprod. Fert.* **85,** 471–476.

Stewart, H.J., McCann, S.H.E., Barker, P.J., Lee, K.E., Lamming, G.E. & Flint, A.P.F. (1987) Interferon sequence homology and receptor binding activity of ovine trophoblast antiluteolytic protein. *J. Endocr.* **115,** R13–R15.

Stewart, H.J., McCann, S.H.E., Lamming, G.E. & Flint, A.P.F. (1989) Evidence for a role for interferon in the maternal recognition of pregnancy. *J. Reprod. Fert., Suppl.* **37,** 127–138.

Vallet, J.L., Bazer, F.W. & Roberts, R.M. (1987) The effect of ovine trophoblast protein-1 on endometrial protein secretion and cyclic nucleotides. *Biol. Reprod.* **37,** 1307–1315.

Vallet, J.L., Bazer, F.W., Fliss, M.F.V. & Thatcher, W.W. (1988) Effect of ovine conceptus secretory proteins and purified ovine trophoblast protein-1 on interoestrous interval and plasma concentrations of prostaglandin F-2α and E and of 13,14-dihydro-15-keto prostaglandin F-2α in cyclic ewes. *J. Reprod. Fert.* **84,** 493–504.

Zoon, K.C. & Wetzel, R. (1984) Comparative structures of mammalian interferons. In *Handbook of Experimental Pharmacology*, vol. 71, pp. 79–100. Eds P. E. Carne & W. A. Carter. Springer-Verlag, New York.