

Evolution of the interferon τ genes and their promoters, and maternal–trophoblast interactions in control of their expression

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It is well established that the interferon τ (IFN- τ) family of proteins play a major role in preventing the regression of the corpus luteum during early pregnancy in ruminants, such as cattle, sheep and goats, but not in other mammals. These interferons, which are structurally and functionally related to type I interferon, such as IFN- α and ω , arose from a duplication of an IFN- ω gene approximately 36 million years ago. The IFN- τ genes have continued to duplicate since that time and have acquired the ability to be transcribed uniquely in the trophectoderm. Low expression is first detectable at the blastocyst stage, but massive transcriptional upregulation occurs a few days later during the initial stages of conceptus elongation. Expression is finally terminated upon trophectoderm attachment to uterine endometrium. The major promoter element that controls expression is an Ets-2/AP-1 enhancer element. Growth factors and cytokines released by the maternal endometrium that, possibly in response to progesterone, act through Ras and the mitogen-activated protein kinase (MAP-kinase) signal transduction pathway have been implicated in controlling IFN- τ gene transcription by activating Ets-2. This timely expression of IFN- τ is not only required to rescue the corpus luteum of pregnancy but may also be an indicator of conceptus fitness, thereby serving as a critical factor that dictates the continuation of pregnancy in ruminants.

Introduction

In most eutherian mammals, the functional lifespan of the corpus luteum has to be extended during pregnancy to provide continued production of progesterone and to maintain the uterine endometrium in a receptive state for conceptus development. The mechanisms used to achieve corpus luteum rescue differ markedly among groups of mammals (Roberts *et al.*, 1996). In cattle, sheep and related pecoran ruminants, the trophoblast does not produce a

chorionic gonadotrophin analogous to hCG, yet the pregnant female must be notified by a chemical signal that a conceptus is present in her uterus even before the trophoblast has attached firmly to the uterine wall. If intervention in the normal ovarian cycle does not occur, the corpus luteum will regress as a result of the action of the luteolytic hormone, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). In a non-pregnant animal, $PGF_{2\alpha}$ is released from the uterine endometrium in a series of pulses of increasing amplitude, beginning late in the luteal phase. For the pregnancy to be successful, the conceptus must prevent luteolysis, and this intervention is accomplished by dampening $PGF_{2\alpha}$ pulsatility (Thatcher *et al.*, 1997).

The anti-luteolytic factor responsible for rescuing the corpus luteum of cattle and sheep was shown in the 1980s to be an interferon, now known as interferon tau ($IFN-\tau$) (Roberts *et al.*, 1997). The manner whereby $IFN-\tau$ intervenes in the normal luteolytic process has been widely reviewed (Spencer *et al.*, 1995; Thatcher *et al.*, 1995; Robinson *et al.*, 1999; Demmers *et al.*, 2001) and will not be discussed further in this study. The discovery that a cytokine was implicated as a hormone of pregnancy (Imakawa *et al.*, 1987, 1989) caused considerable surprise and interest. By that time, interferons had been well established as potent antiviral agents and were beginning to be used therapeutically to treat viral diseases and various forms of cancer (Pestka *et al.*, 1987; DeMaeyer and DeMaeyer-Guignard, 1988), but a role in reproduction was totally unexpected.

Interferon was first described in the 1950s as a result of its ability to protect cells from viral infection, but remained poorly characterized for almost 25 years because the individual proteins are produced in small amounts and are active at very low concentrations (Pestka *et al.*, 1987; DeMaeyer and DeMaeyer-Guignard, 1988). There are two main classes of interferon, still often referred to as type I and type II. $IFN-\gamma$ (type II) is an inflammatory cytokine and the product of a single gene. It has its own unique receptor and is expressed primarily by various sub-populations of activated T-lymphocytes and a restricted number of other types of cell (DeMaeyer and DeMaeyer-Guignard, 1988; Bach *et al.*, 1997). $IFN-\gamma$ is released in relatively large quantities by the pig trophoblast in the peri-implantation period but its functional role during pregnancy remains unknown (Lefevre *et al.*, 1990, 1998).

The type I interferons are an extensive grouping of related genes found throughout vertebrates and include $IFN-\alpha$, $-\beta$, $-\omega$ and $-\delta$, as well as $IFN-\tau$ (Roberts *et al.*, 1998). Most of the sub-types, including $IFN-\tau$, are represented by multiple genes. The three-dimensional structure of their proteins is based on a bundle of five α -helices and a number of inter-helical loops, one of which, the AB loop, interacts extensively with the type I receptor. The hallmark of the entire grouping is their potent antiviral activity, which is usually evident at picomolar concentrations (Alexenko *et al.*, 1997; Ealy *et al.*, 2001). All type I interferons are pleiotrophic in action, typically possessing anti-proliferative and complex immunomodulatory as well as antiviral properties (Pestka *et al.*, 1987; DeMaeyer and DeMaeyer-Guignard, 1988). Subtle differences in properties among individual type I interferons have been widely documented and are probably based on the manner in which each protein interacts with the two subunits of the common receptor to activate intracellular signal transduction pathways (Uzé *et al.*, 1995; Cook *et al.*, 1996; Pestka, 2000). Nevertheless, when comparisons have been made, $IFN-\tau$ possesses the typical biological activities of other type I interferons. Importantly, there is little compelling evidence that $IFN-\tau$ is superior as an anti-luteolytic agent. $IFN-\tau$ differs most markedly from other type I interferons in the lack of inducibility of the IFN genes in response to virus, the localized expression of $IFN-\tau$ genes to one epithelial cell layer (trophoblast), and the very high rate and persistence of $IFN-\tau$ protein production over a few days at a crucial time during pregnancy when the corpus luteum is wavering on the point of regression and responsive to $PGF_{2\alpha}$ (Roberts *et al.*, 1999). It is likely that what sets the $IFN-\tau$ proteins apart from these other interferons and has provided them with the status of hormones of pregnancy

is their production by the conceptus in the required amounts and at the correct time to prevent regression of the corpus luteum.

The following sections discuss how the IFN- τ genes (*IFNT*) appear to have evolved in concert with the emergence of the ruminant ungulates, a particularly successful group of mammals. In addition, as *cis*-regulatory elements generally govern the onset, magnitude and timing of gene expression, the keys to understanding what makes the IFN- τ uniquely different from other interferons are likely embedded in the control elements of their genes. A second focus of this review is on the unique features of *IFNT* promoters and the possible interplay between the conceptus and maternal uterine endometrium in governing transcriptional activity.

Evolution of the *IFNT*

On the basis of base substitution rates, the first IFN- τ gene (*IFNT*) originated by a single duplication from an IFN- ω gene (*IFNW*) about 36 million years ago (Roberts *et al.*, 1997, 1998). The *IFNW* has been calculated to have arisen from an IFN- α gene (*IFNA*) about 130 million years ago, not long after the emergence of mammals. Hence, the *IFNW* is likely to be present in most orders of mammals. However, the emergence of the *IFNT* occurred late in mammalian evolution, well after the separation of artiodactyls (cattle, pigs, camels and their relatives) from perissodactyls (horses, rhinos and their relatives) and shortly after the pecoran ruminant suborder (Ruminantia) emerged from pigs and non-pecoran ruminants (camels, llamas). Consequently, *IFNT*, and the pregnancy recognition system that these genes orchestrate, are confined to the pecoran mammals (cattle, deer, giraffes and their relatives).

As indicated above, the interferons that are most related structurally to IFN- τ are members of the IFN- ω family. The two sub-types share approximately 70% amino acid sequence identity within a species, and both have an additional six amino acids at their carboxyl termini, compared with the shorter IFN- α (Roberts *et al.*, 1971, 1998). Ealy *et al.* (1998) have shown that this carboxyl extension is unnecessary for the anti-viral activity of the IFN- τ and for their ability to extend the oestrous cycle of ewes. The most likely explanation for the longer length of the IFN- ω and - τ is that a common progenitor gene acquired an extra six codons through mutation of a stop codon. Whether this addition of six amino acids contributes to structural stability of the molecules or has some other positive advantage remains unclear.

An examination of *IFNT* and *IFNW* reveals that similarities in gene sequence disappear about 130 bases upstream of their transcription start sites and about 120 bp downstream of their stop codons, limits that might reflect the sites of the original duplication event (for further details, see Alexenko *et al.*, 1997), which presumably occurred by homologous recombination. As there are presently no known principles governing gene duplications, the significance, if any, of the two putative break points is unclear.

There are no obvious viral control elements remaining in the *IFNT* promoters, whereas the *IFNW* lack the intact Ets-2 binding site, which is probably critical for trophoblast-specific expression (see next section). Phylogenetic analysis has been performed on the cDNA sequences of the multiple, expressed *IFNT* from cattle (*Bos taurus*) and sheep (*Ovis aries*) and the single genomic sequences available for musk oxen (*Ovibus moschutus*), goat (*Capra hircus*) and giraffe (*Giraffida camelopardalis*) (Fig. 1). The single red deer (*Cervus elaphus*) cDNA sequence so far described is also included in the scheme, as are the genomic sequences for two *IFNW* from sheep and cattle. It should be emphasized that each of the species represented by only a single gene or cDNA sequence in Fig. 1 probably have many *IFNT* and that these genes could be as divergent as those seen in sheep. Note that the *IFNT* from musk ox and

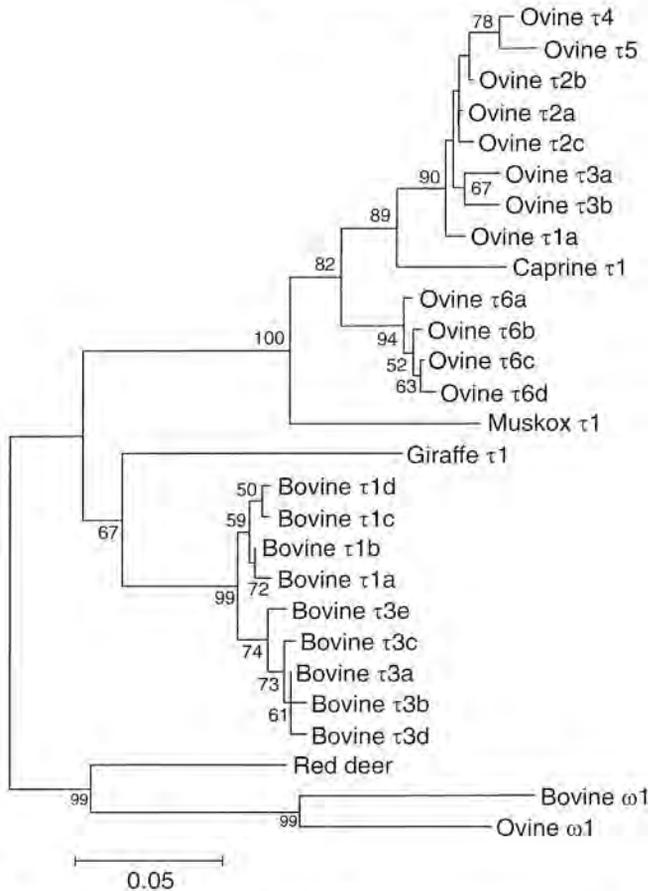


Fig. 1. A phylogenetic tree for the known interferon τ (IFN- τ) proteins. Construction of the tree was by a neighbour joining procedure (Kumar *et al.*, 1993) based on the proportion of amino acid differences between sequences. Signal sequences are not included in the analysis. The numbers of branches are bootstrap percentages (percentage of bootstrap pseudo-samples supporting that branch). Therefore, each branch is shown with a numerical value, reflecting its reliability, except in instances where the bootstrap values were <50% (and hence were highly unreliable). Bovine and ovine IFN- ω are included in this analysis for comparison with the IFN- τ . The lengths of the branches reflect the degrees of amino acid diversity between interferons. The scale bar designates P value of 0.05. The GenBank accession numbers for sequences are M60903, M60908, M60913, M26386, M73243-M73245, AF158818-AF196327, AF270471, Y00287, X56341, X56342, U55050 and AJ000638.

goat cluster with those from sheep, emphasizing the close phylogenetic relationship of these three species. The single giraffe *IFNT* so far cloned is well separated from the cattle and sheep genes, consistent with the early divergence of the Giraffidae from the Bovidae. Even further distanced is the cervid (red deer) cDNA sequence, which appears to display sequence features intermediate between the *IFNW* and *IFNT* of cattle.

Many different IFN- τ cDNA have been sequenced from bovine and ovine conceptuses. In each species they fall into well-defined clusters (Fig. 1). Although it is unclear whether all the multiple forms of ovine and bovine IFN- τ 1 in Fig. 1 represent different genes, as some of the minor variants present may represent polymorphic forms transcribed from the same gene locus, there is abundant evidence to indicate that many closely linked genes are present at the locus (Ryan and Womack, 1993). Moreover, the phylogenetic tree shown in Fig. 1 indicates that the *IFNT* are continuing to duplicate at high rates. The bo*IFNT*, which differ by $\leq 3\%$ in nucleotide sequence, can be calculated to have arisen by a series of recent duplications, all occurring within the last 6–8 million years. Recent data indicate that these genes are under similar transcriptional control (Ealy *et al.*, 2001). It remains unclear why there is a need for so many *IFNT*. One possibility is that the expression of several genes fulfills the requirement for large quantities of IFN- τ protein. Another possibility is that there are strong selective pressures for emergence of 'better' IFN- τ proteins to provide greater fitness to the conceptus (see final section of this paper).

Transcriptional control of *IFNT* expression

As stressed earlier, what sets IFN- τ apart from other type I IFN is their expression in trophoblast and their lack of responsiveness to viral induction (Cross and Roberts, 1991; Ealy *et al.*, 2001). *IFNT* promoter reporter constructs are not inducible by virus, indicating that any viral response elements became disrupted as the *IFNT* evolved. The trophoblast-specific expression of *IFNT* appears to be governed by two specific promoter regions. One region is a distal A/T rich element situated approximately –358 to –322 bp from the transcription start site, which has not been studied in detail. The second is in the proximal region (–91 to –69) of the promoter (Fig. 2). Both of these regions of the *IFNT* form strong associations with nuclear proteins in electrophoretic mobility shift assays performed with extracts prepared from elongating ovine and bovine trophoblast (Leaman *et al.*, 1994). These protein–DNA associations disappear as the *IFNT* become downregulated a few days later. Moreover, the removal of these regions from *IFNT* promoters significantly reduces promoter activity. Deletion of the –91 to –61 nucleotide sequence, in particular, had a major effect, reducing basal promoter activity by $>70\%$ (Ezashi *et al.*, 1998).

Ezashi *et al.* (1998) used four tandem copies of the –91 to –69 region of the *IFNT* promoter as 'bait' (or target) in a yeast single-hybrid system to screen for putative transcriptional regulators in a cDNA library prepared from day 13 elongating ovine conceptuses. The transcription factor, Ets-2, bound this bait sequence, which, upon further examination, was observed to contain a close-to-consensus Ets-2 binding sequence with a central C/AGGAA/T core motif (Figs 2, 3a). Significantly, three ovine *IFNT* that are poorly expressed in ovine conceptuses, and are probably pseudogenes, possess IGAA rather than the conserved GGAA core sequence in this critical region of their promoters and are not Ets-2-responsive (Ezashi *et al.*, 1998).

The choriocarcinoma cell line, JAr, has been used extensively to analyse *IFNT* promoter activity (Cross and Roberts, 1991; Leaman *et al.*, 1994; Ezashi *et al.*, 1998, 2001) because a suitable bovine cell line that can be experimentally transfected is not available. Ets-2 overexpression in JAr cells strongly upregulated bovine *IFNT1* promoters as long as they contained the proximal enhancer region (Fig. 3b). In contrast, when the Ets-binding sequence on the promoter was mutated by changing the core sequence from AGGAA/G to TAGTCG, both basal and Ets-2-enhanced activity was markedly reduced (Fig. 3a,b). Ezashi *et al.* (2001) demonstrated by western blot analysis that Ets-2 is present in JAr cells that have not been transfected with an Ets-2 expression plasmid, and it seems likely that endogenous Ets-2

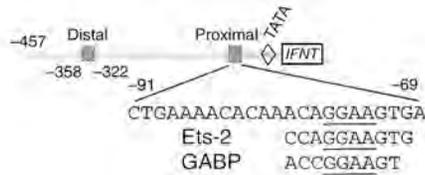


Fig. 2. A diagram of the conserved promoter sequences of the interferon τ genes (*IFNT*). Two regions, one distal and the other proximal, appear to bind transcription factors present in day 13 ovine conceptuses and have been implicated in transcriptional control of promoter expression. The sequence of the proximal element, containing an Ets-2 binding site, is shown. Numbers indicate base position relative to the transcription start site of the gene.

contributes to the basal *IFNT* promoter activity observed in the JAr cell line. Ets-2 protein can also be detected in nuclei of ovine trophoblast cells during the period that IFN- τ is strongly expressed by conceptuses (Ezashi *et al.*, 1998). These data indicate that the Ets-2 interaction with the proximal promoter region of *IFNT* is a limiting factor in IFN- τ expression in trophoblast.

Ets-2, although a widely expressed transcription factor, seems particularly important in controlling differentiation and possibly formation of trophoblast. Not only is it a key transcription factor for several genes characteristically expressed in trophoblast, including *IFNT*, (Ezashi *et al.*, 2001), but also deletion of the murine *ets-2* leads to embryonic death by day 8.5 as the result of defective placental development (Yamamoto *et al.*, 1998). In this context, it should be noted that mice do not possess IFN- τ genes, and that the lethality must be an outcome of effects of Ets-2 on other genes necessary for proper trophoblast development.

The production of IFN- τ by bovine and ovine conceptuses begins at the blastocyst stage, when expression on a per cell basis is very low, and begins to rise as the conceptus enlarges spherically and then begins to expand and elongate through the uterine lumen (Farin *et al.*, 1990; Roberts *et al.*, 1992). Conceptus elongation and hence the onset of maximal IFN- τ production is quite variable from animal to animal and appears to be correlated with the increase in maternal concentrations of serum progesterone, the hormone that controls secretory activity of endometrium (Ashworth and Bazer, 1989). We have speculated that factors in maternal uterine secretions produced in response to increasing progesterone, such as the cytokines granulocyte-macrophage colony stimulating factor (GM-CSF) and colony stimulating factor 1 (CSF-1), might upregulate *IFNT* expression via Ets-2. For example, luteal phase secretions from a day 15 non-pregnant ewe, when added to *in vitro* produced bovine blastocysts, increased the production of IFN- τ approximately threefold during the subsequent 48 h of culture, while having only a modest effect on the number of cells (Fig. 4). Day 12 secretions, at a similar protein concentration, were much less effective. It remains unclear why expression of *IFNT* continues indefinitely in cultured outgrowths of bovine blastocyst (Hernandez-Ledezma *et al.*, 1992), whereas it is transient within the uterus. Attachment of the trophoblast to the uterine epithelium may provide the trigger that silences gene expression (Guillomot *et al.*, 1990).

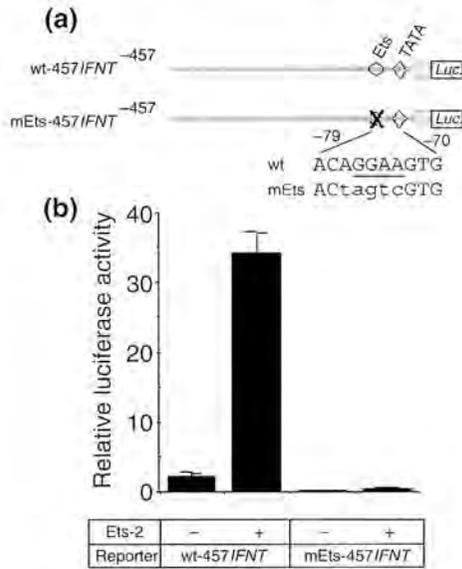


Fig. 3. Ets-2 transactivates an interferon τ gene (*IFNT*) promoter. (a) Sequences of the wild type and mutated Ets-2 binding sequences used in the promoter-luciferase reporter constructs for the transfection experiments are summarized in (b). (b) The wild-type -457 bovine *IFNT* promoter is upregulated about 35-fold by co-transfection with Ets-2 in JAr choriocarcinoma cells. By contrast both basal and Ets-2 stimulated expression from the mutated promoter is almost completely abolished. Similar results have been published by Ezashi *et al.* (1998), who describe the methodology in detail.

An explanation of how such factors in uterine secretions might target the Ets-2 enhancer is currently being tested in this laboratory. It is known that certain Ras family members form an important node between cell surface receptors for certain cytokines and growth factors and downstream MAP-kinase pathways (Fowles *et al.*, 1998; Yordy and Muise-Helmericks, 2000). Constitutively activated Ras, for example, has an ability to increase markedly the transactivation potential of Ets-2 by stimulating the phosphorylation of a highly conserved threonine residue (T72) in the so-called 'pointed' domain (Fig. 5a). In addition, many cellular genes, including the gene for urokinase-plasminogen activator (Stacey *et al.*, 1995; Fowles *et al.*, 1998; Yordy and Muise-Helmericks, 2000), have been shown to contain such Ras-responsive enhancers, which typically have an Ets-2 binding site closely juxtaposed to a binding site for AP1 (Fig. 5b,c). The production of the protease urokinase-plasminogen activator by mouse trophoblast cells temporally coincides with the invasive phase of the embryo and is concentrated in regions of invasion (Harvey *et al.*, 1995; Sharma, 1998). Metalloproteinases, such as stromelysin, are also produced by murine trophoblast (Whiteside *et al.*, 2001). The stromelysin promoter, for example, possesses a Ras responsive enhancer composed of two inverted Ets sites (Fig. 5b). Close examination of the *IFNT* proximal promoter reveals that there

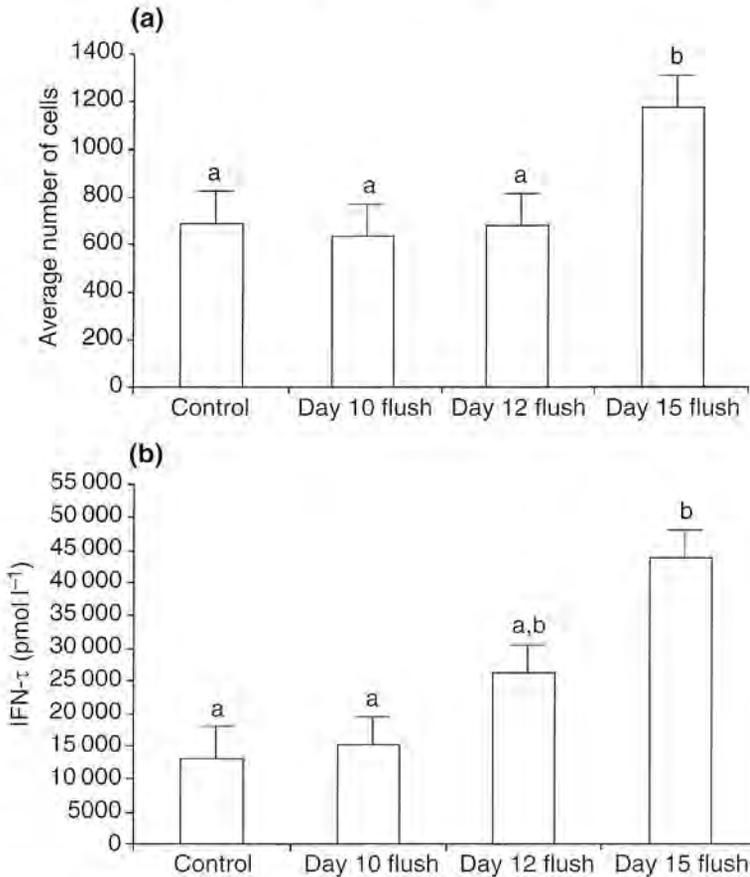


Fig. 4. The effect of addition of proteins present in uterine flushings from non-pregnant sheep at different stages of the oestrous cycle on (a) number of cells and (b) interferon τ (IFN- τ) production from day 9 hatched bovine blastocysts cultured *in vitro* for 48 h. Experimental details, including the production of blastocysts, recovery of uterine flushings, determination of the number of cells and assay of IFN, can be obtained from Kubisch *et al.* (2001). The data in each column of the histogram represent the mean production of IFN- τ \pm standard error from 10 blastocysts that had been cultured in individual microdrops for 48 h. ^{a,b}Where letters above columns differ, values are significantly different ($P < 0.05$). The figure has been amended from Figs 2 and 3 of Kubisch *et al.* (2001) with permission.

is a close-to-consensus AP1 site adjacent to the Ets motif (Fig. 5c). In a series of experiments conducted predominantly on murine 3T3 cell lines (Ezashi and Roberts, 2000), which, unlike JAr cells, do not possess a constitutively active Ras component, it has been demonstrated (i) that an *IFNT* promoter is super-induced by expression of Ras and Ets-2 together, but not by either factor alone; (ii) that mutation of T72 on Ras partially, but not totally, reverses this effect; (iii) that mutation of the adjacent AP1 site also leads to a reduction in the ability of Ras and Ets-2 to transactivate the *IFNT* promoter; and (iv) that CSF-1 is able to upregulate transfected *IFNT*-promoter-reporter constructs in 3T3 cell lines stably expressing the CSF-1 receptor, *c-fms*, but not in lines expressing a mutated receptor.

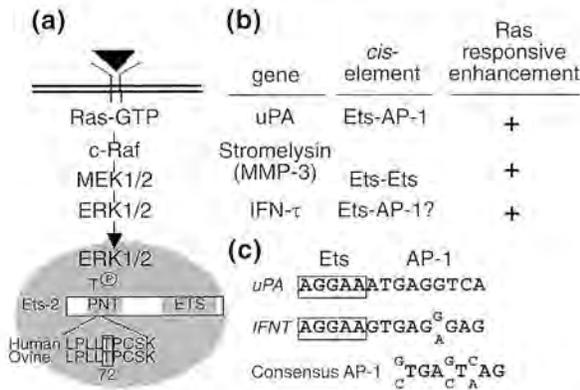


Fig. 5. The Ets-2 enhancer of the interferon τ gene (*IFNT*). (a) The activation of Ets-2 by the Ras/mitogen-activated protein (MAP) kinase pathway is illustrated schematically. An extracellular ligand, such as colony stimulating factor 1 (CSF-1), binds to its receptor, which activates a branch of the MAP-kinase signal transduction pathway. The outcome of these events is the phosphorylation of a conserved Thr72 (boxed) on Ets-2, which is already bound to DNA. This modification within the Ets-2 pointed domain stimulates transcription. (b) Examples of Ets-2 enhancers that are responsive to activated Ras. Ets-2 enhancers vary widely in the manner in which they have evolved to respond to Ets-2. Some are bipartite, for example the matrix metalloproteinase 3 (MMP-3) enhancer; others, including urokinase-plasminogen activator (uPA) and possibly *IFNT*, have an adjacent AP1 site. (c) The putative AP1 site of *IFNT* is different in sequence from that of uPA, but falls within the consensus sequence for such sites.

Together, the above data indicate that the Ets-2 enhancer region of the *IFNT* may be required, not only for trophoblast-specific expression, but also for coordinating the responsiveness of *IFNT* gene expression to the maternal uterine environment.

Interferon signalling: Why has it arisen?

There is great variability within mammals in the gross morphology of the placenta, and in the manner, timing and degree to which the trophoblast breaches the uterine epithelium (Roberts *et al.*, 1996). Therefore, it is perhaps not surprising that different groups of mammals have been forced to evolve disparate signalling mechanisms to accomplish and maintain a dialogue between the mother and her developing offspring.

Although the remarkable differences between species are apparent, it is less clear why they exist. Neither the synepitheliochorial placenta of ruminants nor the epitheliochorial placenta of pigs involve penetration of trophoblast into the endometrium or tapping of maternal blood vessels, yet they are as efficient as the haemochorial placenta of primates at delivering young. Perhaps the best explanation for explaining diversity in placental structure is that it has been driven by genetic conflict. Just as a parasite seeks new ways to avoid host defence mechanisms to exploit its host, the placenta has evolved to maximize benefits to the fetus. The mother, in turn, has devised counteracting measures to limit exploitation and to ensure her own

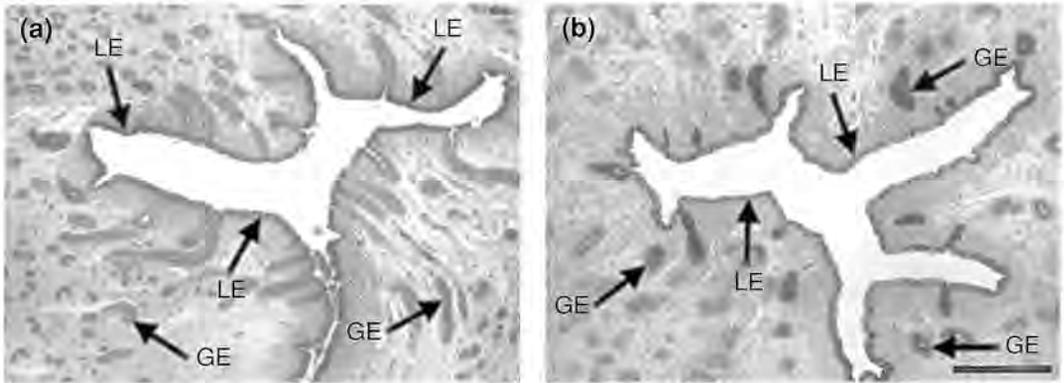


Fig. 6. Immunohistochemical localization for interferon α receptor (IFNAR) 1 and 2 subunits in the uteri of pregnant ewes. (a) Incubated with antiserum against IFNAR1. Arrows depict staining in luminal epithelium and glandular epithelium cells. (b) Incubated with antiserum against IFNAR2. Arrows show staining in luminal epithelium (LE) and glandular epithelium (GE) cells. (a–b) Scale bar represents 200 μm . The figure has been amended from Fig. 2 of Rosenfeld *et al.* (2002) with permission.

interests are protected. This analogy, although not wholly satisfactory because pregnancy exhibits features of both conflict and cooperation between the mother and her fetus, seems to provide the simplest explanation for why the placenta, which essentially has the same function in all mammals, should have diverged so rapidly along different branches of the evolutionary tree. This analogy should not be taken too far. It is, after all, in the interest of the mother and fetus that neither party escalates the conflict to the extent that reproduction is compromised. We speculate that the IFN- τ progenitor gene presumably had an initial role in protection against viral infection and that its conversion to a conceptus signalling agent and the gradual refinement of this role involved key mutations in its promoter, which ultimately permitted expression early in trophoblast development and subsequent massive production in response to maternal stimuli.

The fact that different trophoblast signalling mechanisms have evolved to promote similar outcomes, such as maintaining the production of progesterone from the ovary during early pregnancy, indicates that even if the cascade of events initiated by the different signals are different, the pathways achieve a similar end result. A large number of species release a luteolysin, often from the uterus and in many instances PGF $_{2\alpha}$ to terminate their ovarian cycles, yet only a few species use IFN- τ to prevent such an outcome during early pregnancy. One possible explanation is that multiple luteoprotective pathways ultimately converge on the final key steps that reduce either the synthesis or release of PGF $_{2\alpha}$. One intriguing observation is that, during early pregnancy, human and rat endometrium express several IFN- α -responsive genes (Li *et al.*, 2001), but human and rat conceptuses probably do not produce an IFN. Conceivably, some other cytokine, yet to be described, is responsible for upregulating these genes (Li *et al.*, 2001). Conversely, it would not be surprising if the ruminants, although relying largely on a mechanism dependent upon IFN- τ to prevent luteolysis, might be expected to carry vestiges of other pathways inherited from their ancestors.

If we accept the fact that IFN- τ were co-opted as anti-luteolytic factors in association with the evolution of the synepitheliochorial placenta of the large ruminant species, another puzzle is why so much IFN is produced by the conceptus. Even in short-term culture, a single day 15 ovine conceptus produces 100–250 μg IFN- τ in 24 h (Ashworth and Bazer, 1989; R. M. Roberts, unpublished). Although the concentrations of IFN- τ in the uterus at about day 15 of pregnancy are difficult to estimate, they must far exceed those needed to

saturate the type I IFN receptor (K_d approximately 300 pM) (Hansen *et al.*, 1989; Han *et al.*, 2001), which is located predominantly on the surface and upper glandular epithelium of the maternal endometrium (Fig. 6). Could the massive output at day 15 be largely irrelevant to pregnancy outcome? One argument against this view is that uterine infusion of smaller amounts of recombinant IFN- τ (10 μ g per day, beginning on day 11 and continuing up to day 18) fails to extend luteal lifespan (Winkelman *et al.*, 1999). Another explanation of why so much IFN- τ is produced is that it targets sites outside the uterus, although there is little evidence that it enters the systemic blood circulation, and the majority of receptors are located at sites where they are most likely to intercept IFN- τ released from the conceptus (Fig. 6). There is also the possibility that the antiluteolytic events that are triggered by IFN- τ require locally high concentrations of ligand, either because the signal transduction pathway involved in dampening PGF $_{2\alpha}$ release operates optimally only at full receptor occupancy or because a non-classical receptor with high K_d is involved. Finally, it should not be overlooked that IFN- τ , like hCG in the human, might be monitored by the mother as an indicator of conceptus fitness. Haig (1993) has described hCG as the 'battle cry' of the human embryo. An analogous role for IFN- τ in the ruminant species seems not to be unreasonable.

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