The role of nuclear factor kappa B in human labour

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Abstract

Preterm birth remains the leading cause of perinatal mortality and morbidity, largely as a result of a poor understanding of the precise mechanisms controlling labour onset in humans. Inflammation has long been recognised as a key feature of both preterm and term labour, with an influx of inflammatory cells into the uterus and elevated levels of pro-inflammatory cytokines observed during parturition. Nuclear factor kappa B (NF-κB) is a transcription factor family classically associated with inflammation. Accumulating evidence points to a role for NF-κB in the physiology and pathophysiology of labour. NF-κB activity increases with labour onset and is central to multiple pro-labour pathways. Premature or aberrant activation of NF-κB may thus contribute to preterm labour. The current understanding of NF-κB in the context of human labour is discussed here.

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Introduction

Human labour is the culmination of fetal maturation, fetal membrane rupture, placental separation, cervical ripening and dilatation, and coordinated uterine contractions. The precise mechanisms controlling the onset of labour in humans remains one of the fundamental unsolved questions in reproductive biology. Understanding this process is of great importance given that premature delivery complicates up to 10% of pregnancies and is associated with up to 70% of neonatal morbidity and mortality. Inflammation is now widely accepted to be a key feature of human labour. Microarray and suppression subtractive hybridization studies have demonstrated the up-regulation of a large panel of pro-inflammatory genes in both fetal membranes (Marvin *et al.* 2002, Bethin *et al.* 2003) and myometrium (Chan *et al.* 2002, Charpigny *et al.* 2003) with labour onset. Extensive work over the last two decades has implicated cytokines and prostaglandins in the parturition process. Parturition is characterised by an influx of inflammatory cells into the uterus (Young *et al.* 2002) and increased expression of pro-inflammatory cytokines in both preterm and term labour: elevated levels of interleukin-1β (IL-1β), IL-6 and tumour necrosis factor-α (TNF-α) are detected in amniotic fluid (Romero *et al.* 1992, Opsjln *et al.* 1993, Romero *et al.* 1993, Maymon *et al.* 1999), while IL-1β and IL-6 expression is also increased in amnion (Dudley *et al.* 1996, Keelan *et al.* 1999), myometrium and choriodecidua (Keelan *et al.* 1999, Osman *et al.* 2003) and cervicovaginal secretions (Steinborn *et al.* 1996, Tanaka *et al.* 1998). These

pro-inflammatory cytokines are thought to contribute to labour onset by stimulating the production of IL-8 and prostaglandins. Prostaglandins, in turn, induce uterine contractions and act in concert with IL-8 to cause cervical ripening and fetal membrane remodelling (Olson 2003).

Nuclear factor-kappa B (NF-κB) is a transcription factor family classically associated with inflammation, which is activated in response to infection and pro-inflammatory cytokines, such as those prevalent during labour. Importantly, many pro-inflammatory and labour-associated genes are regulated by NF-κB, and aberrant NF-κB activity underlies a number of inflammation-related disorders. Thus, this transcription factor may play a pivotal role in parturition. NF-κB has been the focus of intense investigation in recent years. Numerous articles have summarized the considerable progress that has been made in elucidating the complexities of NF-κB function and regulation (Hayden & Ghosh 2004, Schmitz *et al.* 2004). This review will briefly outline the regulation of NF-κB and focus on the current understanding of NF-κB activity in the context of human labour.

The NF-κB and IκB proteins

The mammalian NF-κB/Rel family comprises five members: NF-κB1 (p105/p50), NF-κB2 (p100/p52), p65 (RelA), c-rel and RelB (Fig. 1). These proteins share a structurally conserved N-terminal region termed the Rel homology domain (RHD). The RHD mediates dimerization, DNA binding, nuclear localisation and interaction with the

Figure 1 Schematic diagram of NF-κB and IκB family proteins. The NF-κB DNA-binding subunits share a conserved N-terminal rel homology domain (RHD). p65, c-Rel and RelB also have C-terminal transactivation domains (TADs). RelB contains an additional leucine-zipper motif (LZ). The glycine-rich region (GRR) is required for the proteolytic processing of the p105 and p100 precursor proteins, which contain multiple ankyrin repeats, in common with the IκB proteins.

inhibitors of kappa B (IκB) proteins. In the active form NF-κB consists of heterogeneous dimers of various combinations of subunits: each member of the NF-κB family, except for RelB, can form homodimers, as well as heterodimers with one another. RelB forms heterodimers with p100, p52 and p50. The p65, c-rel and RelB proteins contain C-terminal non-homologous transactivation domains (TADs), which are required for transactivation by these proteins. The p50 and p52 proteins are generated by proteolytic processing of their precursors, p105 and p100, respectively. The p105 and p100 precursor proteins are constitutively processed through a cotranslational mechanism, but processing of p105 is much more efficient, so that most cells exhibit constitutively high levels of p50, whereas steady-state levels of p52 are low and tightly regulated (Lin *et al.* 1998, Heusch *et al.* 1999). The p50 and p52 subunits lack TADs, hence homodimers of these proteins have no intrinsic ability to drive transcription and may bind DNA to repress gene expression in resting cells. The prototypical and most prevalent activated form of NF-κB is the p50/p65 heterodimer.

In resting cells, NF-κB is retained in the cytoplasm in an inactive form through association with the IκB proteins, the most prominent of which are IκBα, IκBβ and IκBε. The IκBs are characterized by the presence of multiple

ankyrin repeats, which mediate binding to the RHD of NF-κB (ankyrin repeats; protein motifs containing a 33-amino acid long sequence that occur in tandem array and cooperatively fold into structures that mediate protein-protein interactions). The p105 and p100 precursor proteins also contain ankyrin repeats and can function as IκB-like proteins. Crystallographic and mechanistic studies have revealed that IκBα masks only the nuclear localisation sequence (NLS) of the p65 subunit, leaving the p50 NLS exposed (Huxford *et al.* 1998). This exposed NLS, coupled with nuclear export sequences (NES) present on IκBα and p65, results in constant nuclear-cytoplasmic shuttling of the IκBα/p50/p65 complex. Since the export process is more potent than the import process, the steady-state localization of IκBα-bound NF-κB is in the cytosol (Harhaj & Sun, 1999, Huang *et al.* 2000). Similarly, IκBε also actively shuttles between the nucleus and cytoplasm (Lee & Hannink 2002). In contrast, IκBβ lacks a NES and masks both NF-κB NLSs and IκBβ-bound NF-κB is consequently sequestered in the cytoplasm of unstimulated cells (Malek *et al.* 2001). Two unusual IκB proteins are Bcl-3 and IκBζ. These contain ankyrin repeats but are constitutively nuclear and may act as transactivating transcription factors (Fujita *et al.* 1993, Yamamoto *et al.* 2004).

NF- κ B signalling pathways

Three distinct NF- κ B signalling pathways have been delineated to date, all of which rely on sequentially activated kinases, the generation of DNA-binding NF- κ B dimers, and enhancement of the transcriptional activity of NF- κ B (Fig. 2).

The so-called canonical pathway is triggered by bacterial lipopolysaccharide (LPS) and pro-inflammatory cytokines, such as IL-1 β and TNF α . Whilst many of the very early events following receptor engagement are specific for each stimulus (Hayden & Ghosh, 2004), the signalling converges on the I κ B kinase (IKK) complex (Fig. 3). The IKK complex includes the regulatory scaffold protein NF- κ B essential modulator (NEMO or IKK γ) and the IKK α and IKK β kinases. Once activated, IKK phosphorylates I κ B α on Ser32 and Ser36 (Traenckner *et al.* 1995) and this phosphorylation targets I κ B α for ubiquitination by the SCF ^{β -TrCP} ubiquitin ligase at lysines 21 and 22 (SCF ^{β -TrCP}; The SCF ligase is composed of Skp1,

Cdc53/Cu11, and a specificity-conferring F-box protein, in this case beta-transducin repeat-containing protein; β TrCP) (Yaron *et al.* 1998). Ubiquitinated I κ B α is subsequently degraded by the 26S proteasome, thereby releasing NF- κ B dimers from the cytoplasmic I κ B α /NF- κ B complex, unmasking their NLSs and allowing them to translocate to the nucleus. Canonical NF- κ B activation is usually transient as a result of a 'postinduction repression' mechanism. Due to the presence of NF- κ B binding sites in the promoter of the *I κ B α* gene, activation of NF- κ B leads to the rapid resynthesis of I κ B α , which accumulates in the nucleus and dissociates NF- κ B from DNA-bound complexes (Brown *et al.* 1993). These newly formed I κ B α /NF- κ B complexes are then exported out to the cytoplasm, in a process mediated by the I κ B α NES (Huang & Miyamoto, 2001), which restores the cytoplasmic pool of inactive NF- κ B. I κ B degradation by the canonical pathway requires the IKK β and NEMO components of the IKK

Figure 2 Three distinct NF- κ B signalling pathways. The canonical pathway is activated when tumour necrosis factor- α (TNF α), interleukin-1 β (IL-1 β) or lipopolysaccharide (LPS) bind their respective receptors and involves I κ B kinase (IKK)-mediated phosphorylation of I κ B α . I κ B α is subsequently degraded by the proteasome to release p50/p65 dimers. In the atypical pathway, DNA damage results in extensive phosphorylation of I κ B α by p38-induced casein kinase-2 (CK-2). The noncanonical pathway is triggered by lymphotoxin β (LT β) or B-cell activating factor (BAFF) and involves the activation of IKK α by NF- κ B inducing kinase (NIK). IKK α , in turn, phosphorylates p100, which then undergoes proteasomal processing to generate p52. RelB/p52 dimers are thus released and translocate to the nucleus to activate genes distinct from those regulated by the canonical pathway. 1. Extracellular stimulus 2. Kinase activation 3. Generation and translocation of active NF- κ B dimers 4. DNA-binding and phosphorylation of NF- κ B dimers and cofactor recruitment. P = phosphorylation; Ub = ubiquitination.

Figure 3 Schematic model of the canonical NF- κ B signalling pathway. Stimulation of the cell by tumour necrosis factor- α (TNF α), interleukin-1 β (IL-1 β) or lipopolysaccharide (LPS) leads to the degradation of the small I κ B proteins (classically I κ B α), by the IKK complex. IKK-dependent phosphorylation targets I κ B α for ubiquitination (Ub) and degradation by the proteasome. Once I κ B α is degraded, p65 is phosphorylated (P) and the NF- κ B nuclear localization signals are unmasked, allowing the p50/p65 dimer to translocate to the nucleus where it binds to NF- κ B response elements (κ B) in the promoter of target genes. Induction of p105 degradation may simultaneously occur, thereby releasing further NF- κ B dimers, including p50/p65. The p105 precursor may also be processed in response to cytokines to generate additional p50 homodimers, which may take part in NF- κ B DNA binding or be displaced from DNA by transactivating dimers. In the nucleus, further phosphorylation and acetylation (A) modifications of NF- κ B occur, promoting the recruitment of co-activators, e.g. CREB-binding protein (CBP) to drive transcription. Termination of signalling occurs when newly synthesized I κ B α displaces DNA-bound NF- κ B and exports it back to the cytoplasm.

complex (Tanaka *et al.* 1999, Rudolph *et al.* 2000) and can involve the degradation of I κ B α , I κ B β and I κ B ϵ . The p105 protein may also play a role in the canonical pathway. In addition to the constitutive, cotranslational processing of p105 (Lin *et al.* 1998), which provides the resting cell with the small amount of p50 required for its basal needs (e.g. low-level transcription or repression of transcription), IKK-dependent phosphorylation and ubiquitination of p105 may occur in response to pro-inflammatory cytokines and LPS (Heissmeyer *et al.* 1999, Heissmeyer *et al.* 2001). Such inducible targeting of the p105 precursor can result in two distinct processes, which employ distinct ubiquitin ligases: there may be either complete

degradation or limited processing of the protein (Cohen *et al.* 2004). Presumably processing of p105 will provide solely p50 subunits whereas degradation may release a variety of NF- κ B subunits bound to the p105 ankyrin repeats.

The non-canonical pathway is activated in response to a subset of NF- κ B inducers, such as lymphotoxin β , B-cell activating factor, or the CD40 ligand, although these can also trigger the canonical pathway. In the non-canonical pathway, which is NEMO- and IKK β -independent (Claudio *et al.* 2002, Dejardin *et al.* 2002), IKK α homodimers phosphorylate p100 associated with RelB. This elicits the processing of p100 to p52 and releases transcriptionally

active RelB/p52 dimers (Dejardin *et al.* 2002, Xiao *et al.* 2004). The third pathway is triggered by DNA damage and is considered atypical because it does not involve an initiating receptor ligation event and is independent of the IKK complex. NF- κ B activation by UV irradiation, for instance, involves the multiple phosphorylation of I κ B α on C terminus residues by the p38-activated casein kinase 2 (Kato *et al.* 2003). Whilst all the aforementioned signalling pathways have a requirement for proteasomal degradation of one or more I κ B proteins, a novel pathway involving proteasome inhibitor-resistant (PIR) degradation has recently been described (O'Connor *et al.* 2004). PIR is IKK-dependent and targets I κ B α , but not I κ B β , resulting in constitutive p50/c-rel activity in B cells.

Multiple levels of NF- κ B regulation

NF- κ B is a ubiquitous transcription factor that regulates numerous genes involved in a diverse array of cellular processes. Hence the specificity and temporal control of the NF- κ B response is crucial and multiple levels of control are in place to ensure its tight regulation. As discussed, different stimuli activate distinct kinases, which differentially target I κ B degradation. Since the various I κ B proteins exhibit distinct preferences for association with particular NF- κ B dimers (e.g. I κ B α predominantly binds p50/p65, I κ B ϵ binds p65/c-rel, p100 binds RelB), such selective I κ B degradation can determine whether transcriptionally active or repressive NF- κ B dimers are released. Furthermore, target genes require specific NF- κ B family members for their activation and this varies from gene to gene (Hoffmann *et al.* 2003). Whilst different stimuli can activate distinct NF- κ B dimer combinations, the same stimulus can also elicit distinct waves and subsets of gene activation due to the differential dynamics of I κ B degradation and resynthesis coupled with target gene requirements (Hoffmann *et al.* 2002). Finally, the transcriptional activity of the NF- κ B subunits themselves is also regulated by a variety of post-translational modifications, such as phosphorylation, acetylation, ubiquitination and prolyl isomerization (Schmitz *et al.* 2004). Regulation of NF- κ B signalling is thus complex and cell-type differences in NF- κ B signalling may depend on which kinases are expressed, which subunits of NF- κ B are expressed, in what ratios and on the NF- κ B-activating signals to which a given cell responds.

Insights from mice

A major obstacle in understanding the onset of human labour is the limited ability to extrapolate findings from animal models since there are intrinsic differences between humans and other species in the parturition process, e.g. discordance in the sites and patterns of steroid production. Nevertheless, whilst the role of the corpus luteum and progesterone withdrawal differs between mice

and humans, a number of fundamental similarities are apparent. For instance, both mouse and human exhibit an increase in myometrial cell gap junctions and increased uterine sensitivity to oxytocin at term, as well as similar patterns of cytokine expression during infection-related preterm labour. The primary advantage of using the mouse as a model system is the ability to maintain tight experimental control in an *in vivo* context and to manipulate the mouse genome or proteome in ways that are precluded in human studies due to ethical and practical considerations.

Although genetic disruption of *Nf- κ b* genes has not been informative in terms of their contribution to labour onset, studies using mice deficient in p65 (Alcamo *et al.* 2001), p50 (Sha *et al.* 1995) or c-rel (Donovan *et al.* 1999) have confirmed the requirement of these proteins for the mounting of inflammatory sequelae and the response to infection, both of which impinge on the labour process. The establishment of mouse models of parturition is now beginning to yield interesting findings. A crucial role for NF- κ B in murine parturition was recently demonstrated in a mouse model of term labour (Condon *et al.* 2004). Nuclear translocation of the p50 and p65 subunits in the pregnant mouse uterus increased as term approached. Mice injected intra-amniotically with the NF- κ B inhibitor peptide SN50 displayed a delay in the onset of labour, whereas control mice injected with the inactive SN50mut peptide delivered at term. Furthermore, surfactant protein-A (SP-A), a protein secreted by the fetal lung that reaches maximal levels at term, was shown to increase p65 nuclear levels in the mouse uterus and induce labour. The authors proposed a model wherein SP-A secreted by the maturing fetal lung acts as a trigger for the onset of parturition at term by inducing the migration of macrophages to the maternal uterus, where they activate NF- κ B, resulting in the stimulation of uterine contractility. It is not currently clear to what extent these findings can be extrapolated to the human, although the pattern of SP-A synthesis is similar and a 'fetal signal' to parturition is an attractive hypothesis.

Condon *et al.* (2004) suggest that SP-A signalling to NF- κ B during murine parturition may occur via Toll-like receptors (TLRs). TLRs recognize pathogen-associated molecular patterns in specific classes of microbes and are key regulators of both innate and adaptive immune responses. They share considerable homology with the IL-1 receptors and also signal to NF- κ B through the canonical pathway. Studies using *Tlr-4* $-/-$ mutant mice suggest that this TLR is a critical factor in bacterially-induced preterm labour (Wang & Hirsch 2003). Furthermore, both maternal and fetal polymorphisms of the human *TLR-4* gene have been associated with spontaneous preterm labour (Varner & Esplin 2005). Since NF- κ B is known to mediate TLR-4 signalling, these findings imply a role for NF- κ B in both spontaneous preterm labour, infection-associated preterm labour and possibly normal labour at term.

Suppression of NF- κ B during pregnancy – NF- κ B/steroid antagonism

Suppression of NF- κ B activity during pregnancy might be expected if NF- κ B does play a role in the onset of labour. McCracken *et al.* (2004) reported that NF- κ B down-regulation in T cells of pregnant women is essential for maintaining the cytokine profile necessary for pregnancy success. Suppression of NF- κ B activation in first trimester decidua has also been suggested to contribute to the immunosuppressive mechanisms that prevail during pregnancy (King *et al.* 2001).

Steroids are present in the circulation at high concentrations throughout gestation and are known to have immunosuppressive effects. The steroid hormone progesterone maintains the uterus in a quiescent state for the majority of pregnancy. In most animal species, a withdrawal of this progesterone effect, manifest as a decline in maternal circulating progesterone levels, precedes the onset of labour at term. Although this does not occur in human pregnancy, the administration of antiprogestins to pregnant women induces labour and enhances sensitivity to oxytocin (Chwalisz 1994). Therefore, in the absence of a demonstrable fall in maternal plasma or tissue progesterone concentrations, it has been postulated that alterations in the functions of the progesterone receptor (PR) may mediate functional or local progesterone withdrawal. In human amnion epithelial cells, a transient transfection system was used to demonstrate mutual antagonism between PR and NF- κ B transcriptional activities (Allport *et al.* 2001). Progesterone itself will also repress IL-1 β -induced expression of NF- κ B-regulated genes in human amnion (Loudon *et al.* 2003). This suggests that progesterone may act via its receptor to suppress NF- κ B signalling during pregnancy and that the converse, NF- κ B mediated repression of PR, may occur in association with labour. A negative direct protein-protein interaction between p65 and PR has been demonstrated in breast cancer cells (Kalkhoven *et al.* 1996), while, in a preliminary report, Condon *et al.* (2005) argue that increased p65 expression at term may promote a change in PR isoform predominance, leading to alleviation of the progesterone effect. Progesterone can also bind directly to the oxytocin receptor (OTR) to inhibit its signalling (Grazzini *et al.* 1998). OTR is a regulator of uterine contractility and contains putative NF- κ B binding sites in its gene promoter.

Placental production of glucocorticoids is also markedly increased during pregnancy. The placenta itself is a glucocorticoid-responsive organ and Rosen *et al.* (1998) demonstrated that NF- κ B activity in cytotrophoblasts isolated from human term placenta is chronically suppressed by treatment with the glucocorticoid dexamethasone. Immunohistochemical analysis of cervical tissue from postpartum women compared to that from women at term revealed a decrease in glucocorticoid receptor (GR) levels and a concomitant increase in NF- κ B nuclear localisation

at parturition (Stjernholm-Vladic *et al.* 2004). Thus GR-mediated anti-inflammatory mechanisms may also be in place to suppress NF- κ B activity and ensure pregnancy maintenance. Such inhibitory mechanisms can occur either through p65/GR protein interactions (Garside *et al.* 2004) or via the induction of I κ B α synthesis (Auphan *et al.* 1995).

NF- κ B activity in human labour

In the study of human labour, there have been no genomic or associative genetic reports directly linking NF- κ B to labour onset. However, activation of NF- κ B as a result of alterations in the mechanisms that regulate NF- κ B may occur with labour and aberrant or premature NF- κ B activation could contribute to preterm labour. Research in humans has focused on (i) determining whether the expression or activity of NF- κ B proteins increase within the uterus in association with labour, (ii) investigating the activation of NF- κ B by potential triggers of parturition and, (iii) assessing the requirement of pro-labour pathways for NF- κ B-mediated transcription.

NF- κ B activation in association with labour

The expression profile and activation of NF- κ B family members in myometrial tissue from nonpregnant, term pregnant and spontaneously labouring women was recently reported (Chapman *et al.* 2004). p65, c-rel, p50, p100 and p105 proteins were detected in tissues from all three groups. Surprisingly, a decrease in p65 protein expression was observed in lower segment myometrium in association with labour, although there was no change in fundal myometrium, which is considered the principal contractile region of the myometrium during labour. This decrease was proposed to occur via the selective recruitment of the proteasome complex to a subset of p65-regulated (possibly quiescence-associated) promoters, although this remains to be demonstrated. Nevertheless, NF- κ B is generally post-translationally regulated and a shift in subunit composition of the predominant DNA-binding complex was detected, such that binding of potentially repressive p50 homodimers prevailed in pregnant myometrium, whereas p50/p65 heterodimers were dominant in pregnant and labouring myometrium. However, there has recently been a preliminary report (currently in abstract form) of an increase in nuclear and cytosolic p65 expression in fundal uterine segment myometrium in association with human labour (Condon *et al.* 2005). Increased levels of nuclear NF- κ B at parturition have been demonstrated in the cervix (Stjernholm-Vladic *et al.* 2004).

In amnion, basal and IL-1 β -induced NF- κ B DNA binding activity involves the p50/p65 heterodimer, as well as p65 and p50 homodimers (Lee *et al.* 2003). Allport *et al.* (2001) demonstrated that baseline NF- κ B DNA binding and transcriptional activity of a NF- κ B reporter are

increased in amnion cells following spontaneous labour relative to amnion cells at term, suggesting that NF- κ B plays a role in labour in the amnion. Constitutive NF- κ B activity in many cancer cell types is maintained through continuous I κ B α turnover, as a result of unrelenting IKK activity (Geleziunas *et al.* 1998, Carter *et al.* 2001). However, the increased constitutive NF- κ B activity seen in post-labour amnion cells was paralleled by an increase in I κ B α expression, suggesting that I κ B α is responding to, but not regulating, NF- κ B activation (Allport *et al.* 2001). This apparent paradox is not unprecedented and has been explained by a chaperone-like function of I κ B β . Following stimulus-induced degradation, newly synthesized I κ B β accumulates in the nucleus in a hypophosphorylated form, which can bind NF- κ B without masking its NLS (Suyang *et al.* 1996). Hypophosphorylated I κ B β may thus maintain persistent NF- κ B activity by preventing its sequestration by I κ B α and export to the cytoplasm (Thompson *et al.* 1995, DeLuca *et al.* 1999). Such an I κ B β -mediated mechanism of sustained NF- κ B activity may be responsible for the labour-associated constitutive NF- κ B activity observed in the amnion, since I κ B β expression increases with gestational age and labour onset and there is an increase in nuclear localisation of I κ B β in post-labour cells (Lee *et al.* 2003). However, the phosphorylation status of this nuclear I κ B β , as well as its possible participation in a ternary complex with NF- κ B/DNA, remains to be assessed.

Findings contradictory to the study by Allport *et al.* (2001) were reported by Yan *et al.* (2002a) who showed, by immunohistochemical analysis, that p65 is localised principally in the cytoplasm of amnion and chorion at term, regardless of labour status. However, this study did not examine potential changes in posttranslational modifications of p65 (e.g. phosphorylations, acetylations), which are important for its transactivation potential (Schmitz *et al.* 2004). It is possible that, while a significant increase in nuclear protein levels may not be detectable, labour-associated modifications of pre-existing low-level nuclear p65 could enhance its transcriptional activity. An increase in nuclear localisation of p65 in the decidua was observed with advancing gestation, which was unaffected by labour onset (Yan *et al.* 2002a).

A number of confounding factors may complicate studies of this nature. Challis and colleagues have proposed that functional regionalization of both chorion and myometrium occurs at term, as a result of progesterone action (Challis *et al.* 2000). Thus in the cervical, but not the fundal, region of the chorion there is increased production of prostaglandin E₂ and matrix remodelling. In the myometrium, functional progesterone withdrawal in the fundus induces the expression of contraction-associated proteins (CAPs) and myometrial activation, whereas maintained progesterone signalling in the lower segment myometrium drives the expression of genes that promote relaxation. There may also be inner/outer polarity of the myometrium (Brosens *et al.* 2002). Discrepancies between

studies may therefore reflect the different regions of tissue sampled. Furthermore, Challis *et al.* (2002) have proposed the existence of four phases of uterine activity during pregnancy and labour: a relatively quiescent phase of pregnancy, an initial 'activation' phase of parturition in which mechanical stretch and uterotrophins lead to up-regulation of CAPs and agonist receptors, followed by a 'stimulation' phase in which the uterus responds to uterotonins such as prostaglandins and oxytocin, and a final phase corresponding to postpartum involution. Differences in proximity to labour of the patients classified as not in labour may thus impact on the findings in different studies. The point at which NF- κ B is activated in the parturition cascade remains to be elucidated.

Uterine distension

Stretch is imposed on the myometrium and fetal membranes by the growing fetus throughout pregnancy and more acutely at the time of labour. Studies using unilaterally pregnant rats (Ou *et al.* 1997) suggest that uterine distension is a major regulator of smooth muscle contractility, which may explain the high rate of preterm labour in conditions such as polyhydramnios and multiple pregnancies. Stretch is also likely to contribute to labour at term: decline in uterine growth relative to fetal growth near the end of pregnancy would increase uterine wall stretch and hence promote myometrial activation (Lye *et al.* 2001). The OTR plays an important role in uterine contractions during labour (Romero *et al.* 2000), and OTR mRNA expression is up-regulated by mechanical stretch in pregnant uterine myocytes *in vitro* (Terzidou *et al.* 2005). The OTR promoter contains three putative NF- κ B binding sites. However, although NF- κ B proteins do bind to the OTR promoter and may synergise with the transcription factor CCAAT/enhancer binding protein β to induce OTR transcription in myometrium (Terzidou *et al.* 2003), Johnson and colleagues demonstrated that myometrial NF- κ B DNA binding activity is not stimulated by stretch and therefore does not mediate stretch-induced up-regulation of OTR expression (Terzidou *et al.* 2005). In amnion cells, however, stretch does activate NF- κ B, which in turn induces expression of cyclo-oxygenase-2 (COX-2), an enzyme important in the production of prostaglandins, see below (Mohan *et al.* 2005).

Corticotrophin-releasing hormone

Corticotrophin-releasing hormone (CRH) is a peptide hormone that functions in the hypothalamic–pituitary–adrenal axis to co-ordinate the neuroendocrine response to stress. CRH is also produced by a number of tissues outside the central nervous system. During pregnancy, the placenta and fetal membranes produce large amounts of CRH, whose levels in maternal and fetal blood and in amniotic fluid peak at term (McLean & Smith 2001). Placental production of CRH has been linked with the

timing of birth and Roger Smith's group have proposed a 'placental clock' hypothesis, in which the rate of increase of maternal plasma CRH throughout pregnancy influences the timing of labour (McLean *et al.* 1995).

Thus, in addition to fetal lung SP-A (Condon *et al.* 2004) and bacterial infection (Romero *et al.* 2002), CRH is also considered a possible trigger for the onset of labour. Whereas numerous studies demonstrate a link between NF- κ B activation and either SP-A (Koptides *et al.* 1997, Condon *et al.* 2004) or infection (Lappas *et al.* 2003), there is little information on a possible link between CRH and NF- κ B. CRH may exert paracrine or autocrine actions in uterine tissues, in addition to its endocrine effects. Pro-inflammatory local effects of CRH have been demonstrated in a model of aseptic inflammation in rats (Karalis *et al.* 1991), and NF- κ B was recently shown to mediate the pro-inflammatory effects of CRH in mouse thymocytes (Zhao & Karalis, 2002). The identification of CRH and its receptors in myometrium (Clifton *et al.* 1998, Stevens *et al.* 1998) suggested that CRH may have a direct effect on this tissue. In a study using myometrial explants, CRH was unable to stimulate pro-inflammatory cytokine production (Sehringer *et al.* 2000). However, only lower segment myometrium was examined and CRH is thought to promote relaxation in the lower segment but stimulate myometrial activity in the fundus (Stevens *et al.* 1998). CRH is also expressed in fetal membranes, where it may stimulate prostaglandin production (Jones & Challis, 1989). NF- κ B is an important regulator of prostaglandin production – as discussed below – but a potential link between CRH and NF- κ B activation in fetal membranes has not been addressed.

NF- κ B is activated by and regulates pro-inflammatory cytokines

The importance of pro-inflammatory cytokines in parturition has long been appreciated. A polymorphism in the *TNF- α* promoter that increases *TNF- α* gene transcription is associated with preterm birth in black women, whilst an *IL-6* gene promoter polymorphism that results in reduced *IL-6* production is associated with a decreased risk of preterm birth in Caucasian women (Varner & Esplin 2005). Concentrations of *IL-1 β* , *IL-8*, *IL-6* and *TNF- α* are elevated in amniotic fluid and uterine tissues in association with preterm and term labour (Keelan *et al.* 2003) and mediate infection-induced preterm labour (Romero *et al.* 2002). Furthermore, systemic administration of *IL-1 β* can induce preterm parturition in pregnant mice (Romero *et al.* 1991). The high levels of pro-inflammatory cytokines detected in uterine tissues may derive, in part, from infiltrating leukocytes (Young *et al.* 2002, Osman *et al.* 2003), but decidual, myometrial, amnion and placental cells can themselves all produce pro-inflammatory cytokines. NF- κ B is highly inducible by pro-inflammatory stimuli. LPS (Lappas *et al.* 2002), *IL-1 β* (Belt *et al.* 1999) and *TNF- α* (Kniss *et al.* 2001) have all been shown to stimulate

NF- κ B activity in uterine tissues. In addition, many genes encoding pro-inflammatory cytokines, such as *TNF- α* , *IL-1 β* , *IL-8* and *IL-6*, contain NF- κ B recognition elements within their promoters and NF- κ B is known to promote the formation of cytokines in many cell types. Cytokine-induced NF- κ B can therefore precipitate a positive feed-forward loop resulting in amplification of cytokine production and further NF- κ B activation.

Uterine NF- κ B expression and activity is reduced in *IL-1 β* -deficient pregnant mice compared to pregnant wild-type mice. This is associated with a decrease in cytokine production in response to LPS (Reznikov *et al.* 2000), indicating an important role for NF- κ B in the inflammatory response in uterine tissues. Repression of NF- κ B DNA-binding by the anti-inflammatory agent sulfasalazine (SASP) or the anti-oxidant *N*-acetyl-cysteine (NAC) inhibits the release of *IL-6*, *IL-8* and *TNF- α* from LPS-treated placental, choriodecidual and amnion explants (Lappas *et al.* 2002, Lappas *et al.* 2003). Soloff *et al.* (2004) recently reported the *in situ* binding of the p65 NF- κ B subunit to the endogenous *IL-8* promoter in *IL-1 β* -stimulated myometrial cells. Reporter studies in amnion and cervical cells demonstrated that the NF- κ B response element is required for transcription of the *IL-8* gene in these cells (Elliott *et al.* 2001).

NF- κ B is required for prostaglandin synthesis

Prostaglandins (PGs) are pivotal to the parturition process, mediating cervical ripening and dilatation and stimulating myometrial contractions (Olson 2003). An increase in PG output precedes the onset of clinical labour (Romero *et al.* 1996, Brown *et al.* 1998) and, importantly, drugs that inhibit PG production have been used clinically to attenuate the progression of labour (Besinger *et al.* 1991), whilst the administration of exogenous PGs induces labour and delivery (Ray & Garite 1992). The rate-limiting and committing step of PG synthesis is catalysed by the cyclooxygenase (COX) enzymes. The COX enzymes exist as two main isoforms, the constitutively expressed COX-1 and the inducible COX-2 isozyme (a third isoform, COX-3 has recently been described; Berenbaum 2004). Studies in *Cox-1* $-/-$ knockout mice show that maternal *Cox-1* is necessary and sufficient for murine labour at term (Gross *et al.* 1998). *Cox-2* knockout mice exhibit impaired ovulation and blastocyst implantation, which limits the information that can be gained about the role of COX-2 in murine parturition (Lim *et al.* 1997). However, Gross *et al.* (2000) demonstrated that COX-2 but not COX-1 is induced during LPS-mediated preterm labour, and that administration of a COX-2 selective inhibitor was able to prevent preterm delivery. It has been proposed that COX-1-derived prostaglandins are responsible for the induction of luteolysis, while COX-2 may be important in the final pathway of parturition (Tsuboi *et al.* 2000).

In human labour, which does not involve PG-mediated luteolysis, there is substantial evidence for the importance

of the inducible COX-2. Amnion is a major source of PGs and displays a marked increase in synthesis of PGE₂ with labour onset (Bennett *et al.* 1993). This is associated with the selective induction of the COX-2 gene (Slater *et al.* 1995). Similarly, chorio-decidual cells exhibit labour-associated upregulation of COX-2, but not COX-1, expression (Slater *et al.* 1998). Furthermore, COX-2-selective inhibitors may delay premature delivery in women (Mitchell & Olson 2004).

COX-2 expression in uterine tissues is transcriptionally induced by pro-inflammatory cytokines, such as IL-1 β and TNF α (Belt *et al.* 1999, Kniss *et al.* 2001). The promoter region of the human COX-2 gene contains two NF- κ B response elements and site-directed mutation of either of these elements represses the activity of a COX-2 promoter reporter construct in amnion epithelial cells (Allport *et al.* 2001). Blocking the nuclear translocation of NF- κ B with the SN50 inhibitor peptide results in inhibition of IL-1 β -induced COX-2 protein synthesis in amnion mesenchymal cells (Yan *et al.* 2002b). NF- κ B is also required for TNF α -induced COX-2 expression in trophoblast cells (Kniss *et al.* 2001). Conversely, Lappas *et al.* (2004) reported that inhibition of NF- κ B DNA binding by SASP did not affect COX-2 expression in LPS-stimulated amnion, placenta or chorion. However, SASP is not a specific NF- κ B inhibitor and the authors suggest that SASP could potentially extend the half-life of COX-2. NF- κ B inhibition by SASP does, however, repress secretory type II phospholipase A₂ (an enzyme upstream of COX-2 in the PG biosynthetic pathway) in gestational tissues (Lappas *et al.* 2004), as does NF- κ B inhibition by NAC (Lappas *et al.* 2003). The *in situ* binding of the p65 NF- κ B subunit to the COX-2 promoter has been demonstrated in IL-1 β -stimulated myometrial cells by chromatin immunoprecipitation (Soloff *et al.* 2004). Treatment of myometrial cells with proteasome inhibitors, a COX-2-derived PG metabolite, or a more selective IKK β inhibitor, all of which block NF- κ B activity, inhibits IL-1 β -induced COX-2 expression and PG synthesis (Belt *et al.* 1999, Lindstrom & Bennett 2005).

NF- κ B regulates matrix metalloproteinase expression

Extensive remodelling of the extracellular matrix (ECM) occurs in several processes during parturition, including fetal membrane rupture (Bryant-Greenwood & Yamamoto 1995), cervical ripening (Kelly 2002) and placental detachment from the decidua (Tsatas *et al.* 1999). Fetal membranes consist of amnion and chorion layers connected by ECM, which forms the structural framework of the amnion-chorion. ECM collagens provide the main tensile strength of the fetal membranes. As gestation progresses, programmed collagenolysis takes place, which is mediated by matrix metalloproteinase (MMP) enzymes. A decrease in the collagen content of the membranes and activation of MMPs occurs during labour (Hampson *et al.* 1997, Goldman *et al.* 2003), and inappropriate or

premature MMP expression and activity is proposed to contribute to the pathological preterm premature rupture of membranes (PPROM) (Vadillo-Ortega & Estrada-Gutierrez 2005). ECM collagen degradation during cervical ripening occurs in order to allow the cervix to become soft and distensible, thereby accommodating the passage of the fetus during labour. Increases in MMP activity parallel the increase in cervical dilatation (Winkler *et al.* 1999) and, as with PPRM, premature cervical ripening is a feature of preterm labour (Schmitz 2004).

The onset of labour is associated with an influx of inflammatory cells into the membranes, decidua and cervix (Osman *et al.* 2003), and MMPs found within the uterus may derive from these infiltrating leukocytes (Vadillo-Ortega & Estrada-Gutierrez 2005). The chemokine IL-8 is largely responsible for the influx of leukocytes into uterine tissues, where it induces their degranulation and release of MMPs (Osmers *et al.* 1995). MMPs are also produced by the cervix itself (Stygar *et al.* 2002), as well as the placenta and fetal membranes (Xu *et al.* 2002). The production of MMP-9 (Yoo *et al.* 2002), MMP-1 and MMP-3 (Chase *et al.* 2002), and MMP-2 (Yoshida *et al.* 2001) by inflammatory cells requires the activation of NF- κ B. Lappas *et al.* (Lappas *et al.* 2003) have shown that inhibition of NF- κ B DNA binding by NAC results in the repression of LPS-stimulated MMP-9 activity in amnion and chorion explants.

NF- κ B – a therapeutic target in preterm labour?

Preterm labour constitutes a syndrome, in that it is the outcome of multiple aetiologies. However, uterine inflammation may represent a final common pathway, mediating labour onset resulting from bacterial infections (Romero *et al.* 2002), SP-A secretion from the fetal lung (Condon *et al.* 2004), mechanical stretch (Mohan *et al.* 2005), or genetic predisposition (Varner & Esplin 2005). NF- κ B has long been recognised as a key player in the inflammatory response and work over the last six years has revealed a role for NF- κ B in labour-associated inflammation: NF- κ B is activated by stimuli that induce labour and in turn regulates the expression of molecules involved in the parturition process. Aside from inflammation, a decline in responsiveness to progesterone is also crucial to labour onset in the human, and NF- κ B may antagonise PR to bring about a 'functional' progesterone withdrawal. NF- κ B is thus central to multiple pathways involved in the onset of labour (Fig. 4) and as such constitutes a potential therapeutic target in the management of preterm labour. Treatment directed against NF- κ B has the potential to be more effective than current treatments, which do not cast a sufficiently wide therapeutic net and may also have the added advantage of preventing detrimental effects to the fetus that may result from infection/inflammation (Cornette 2004). However, because of its widespread physiological role, the identification of labour-specific NF- κ B signalling

Figure 4 NF- κ B is central to labour-associated pathways. LPS, lipopolysaccharide; IL-1 β , interleukin-1 β ; TNF α , tumour necrosis factor- α ; CRH, corticotrophin releasing hormone; SP-A, surfactant protein-A; PR, progesterone receptor; GR, glucocorticoid receptor; PLA₂, phospholipase A₂; COX-2, cyclo-oxygenase-2; MMPs, matrix metalloproteinases; IL-8, interleukin-8; OTR, oxytocin receptor.

components and selective targeting of the NF- κ B pathway will be crucial in minimising the systemic toxicity of potential new therapies for preterm labour. The effectiveness of such a strategy may, in part, depend on how early in the parturition process NF- κ B is activated and on the development of improved diagnostic indicators of preterm labour, so that intervention can arrive before a branching cascade has taken hold.

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