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## *Review*

# NF-κB function in the human myometrium during pregnancy and parturition

**Victoria J. Cookson and Neil R. Chapman**

Human Parturition Research Group, Academic Unit of Reproductive and Developmental Medicine, The Department of Human Metabolism, University of Sheffield, The Jessop Wing, Tree Root Walk, Sheffield, South Yorkshire, United Kingdom

**Summary.** Interactions between the nuclear factor kappaB (NF-κB) family of proteins (RelA, RelB, c-Rel, p50 and p52) and DNA are vital for cells to function normally; for example, in the human myometrium, NF-κB-regulated pro-inflammatory mediators, including TNFα, IL-1ß, IL-8 and COX-2 are associated with the onset of labour. NF-κB, however, regulates the expression of over 400 genes, although it is unlikely these would all be activated in concert by a single inducer. At present, defining the role of the NF-κB RelA:p50 dimer, which governs a number of inflammatory promoters, is at the forefront of the parturition research field. However, to over-look the function of other family members and how they may regulate alternative signalling networks within reproductive tissues, only serves to ensure we will never fully understand the molecular circuitry influenced by this family of transcription factors. Consequently this review highlights other mechanisms by which the NF-κB family of regulators have been shown to function in other systems and how they may readily translate to understanding the regulation underpinning human parturition.

**Key words:** Gene Regulation, Inflammation, NF-kappaB, Parturition, Transcription, Premature birth

*Offprint requests to:* Neil R. Chapman, Human Parturition Research Group, Academic Unit of Reproductive and Developmental Medicine, The Department of Human Metabolism, University of Sheffield, The Jessop Wing, Tree Root Walk, Sheffield, South Yorkshire, S10 2SF, United Kingdom. e-mail: n.r.chapman@sheffield.ac.uk

### Premature labour

In the developed world, premature birth complicates 6-10% of pregnancies (Lumley, 2003). As a nation, England has the highest incidence of premature birth in Europe with 42,500 pre-term deliveries recorded in 2003-2004 (www.tommys.org/media/statistics/, Maternity Statistic, England, Department of Health May 2003-2004). In the USA, 500,000 preterm births were recorded in 2003-2004 (Martin et al., 2005), while hospital charges for 25,000 infant admissions with a primary diagnosis of premature birth totalled $1.9 billion in 2003 (www.marchofdimes.com/prematurity/21219.asp). Significantly, the incidence of birth before 28 weeks gestation (severely preterm) is increasing with those infants having elevated risks of major long-term mental and physical handicap (Marlow et al., 2005). Moreover, such infants have a disproportionate effect on health-care budgets world-wide: a recent UK estimate of the total cost of preterm birth to the public sector was £2.95 billion (Mangham et al., 2009). Current tocolytic therapies have limited use and are associated with complications for both infant and mother (Oei, 2006). Since the antenatal health of a baby is seen as a major predictor of adult morbidity (Gill, 2000), reducing the incidence of premature birth is paramount considering the soaring costs of health care for such adult diseases.

### NF-κB and parturition

In humans, a number of receptors, pro-inflammatory cytokines and inducible factors, associated with the onset of both normal and preterm labour, are regulated by NF-κB including TNFα, IL-1ß, IL-8, COX-2, oxytocin receptor and phospholipase-A2. These have been examined in a variety of gestational tissues

including amnion and decidua (Allport et al., 2001; Elliot et al., 2001; Yan et al., 2002a;b; Lappas et al., 2003; Lee et al., 2003; Mohan et al., 2007), cervix (Elliot et al., 2001; Lappas et al., 2004; Lappas and Rice 2004; Stjernholm-Vladic et al., 2004) and myometrium (Belt et al., 1999; Chapman et al., 2004, 2005; Condon et al., 2004, 2006; Soloff et al., 2004, 2006; Lindström and Bennett, 2005; Hardy et al., 2006; Terzidou et al., 2006; Lindström et al., 2008). Furthermore, inhibitors of NF-κB activity, including IL-10 (Schottelius et al., 1999), can suppress both LPS-induced pre-term labour in mice and rats (Sato et al., 2003) while anti-inflammatory prostaglandins (PG), namely PGJ, can repress NF-κB-induced COX-2 expression in cultures of human smooth muscle cells (Lindström and Bennett, 2005). Moreover, IL-1ß-induced uterine contractions in Rhesus monkeys (Sadowsky et al., 2003) can also be suppressed by IL-10, providing further, albeit circumstantial, evidence for NF-κB function during parturition. The following short review will focus on current knowledge of how NF-κB functions to govern myometrial gene expression. The reader is directed to an excellent recent review (Lappas and Rice, 2007) for a more general critique of NF-κB signalling during human labour.

## NF-κB biology

Nuclear Factor-kappaB (NF-κB; Fig. 1A), which is rapidly induced by over 400 different stimuli including TNFα and bacterial lipopolysaccharide (LPS) (Perkins, 2007; also see for an up to date list of known NF-κB inducers: www.nf-kb.org/), is present in virtually every cell type within the body. At present, 11 dimeric complexes of NF-κB have been identified under physiological conditions: these are formed from five distinct subunits: RelA (p65), RelB and c-Rel (which contain transactivation domains within their C-termini) and NF-κB1 (p105/p50) and NF-κB2 (p100/p52) which undergo proteolysis to yield the DNA-binding isoforms, p50 and p52 which lack transactivation domains (Fig. 1B; Chapman et al., 2002). Consequently, homo- and heterodimeric complexes which harbour RelA, c-Rel or RelB are generally transcriptionally active while those homodimers of p50 or p52 are viewed as transcriptionally inert since they still have DNA-binding capability but cannot activate gene expression (Perkins, 2007). Indeed, recent data to support this notion is that even in un-stimulated U937 monocytic cells certain NF-κB subunits; in particular p50 may occupy promoter sites (Schreiber et al., 2006). There are, however, clear

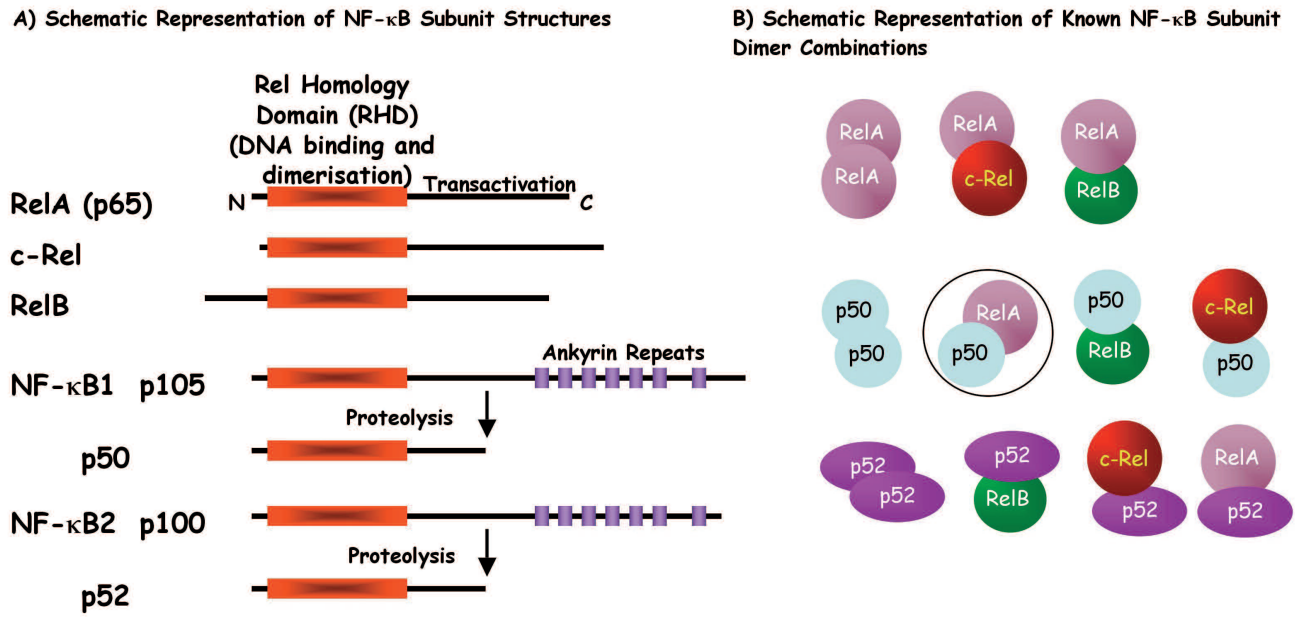


**Fig. 1.** Schematic of NF-κB subunits and dimer combinations. **A.** NF-κB dimers are formed from five distinct subunits: RelA (p65), RelB and c-Rel, NF-κB 1 (p105/p50) and NF-κB 2 (p100/p52). All subunits posses a N-terminal domain of approximately 300 amino acids termed the Rel Homology Domain which mediates both DNA binding and subunit dimerization. RelA (p65), RelB and c-Rel, all contain transactivation domains within their C-termini which, in general, permit dimers containing these subunits to induce gene expression. This domain is also the site of multiple regulatory post-translational modifications including phosphorylation, acetylation and nitrosylation. In contrast to this, NF-κB1 (p105/p50) and NF-κB2 (p100/p52) which undergo proteolysis to yield the DNA-binding isoforms, p50 and p52 lack transactivation domains. Consequently, those homodimers of p50 or p52 are generally viewed as transcriptionally inert since they still have DNA-binding capability but can not activate gene expression: there are, of course, exceptions to this highlighted in the main body of the text. **B.** At present, 11 dimeric complexes of NF-κB have been identified under physiological conditions. The most commonly studied being the RelA:p50 dimer (circled) through its close association with the inflammatory response.

exceptions to this consensus: complexes of both p50 or p52 homodimers and Bcl3 are known to be transcriptionally active in a variety of cell lines (Bours et al., 1993; Fujita et al., 1993) whilst removal of DNA-bound p50 homodimers by Bcl3 has also been reported to induce transcription (Franzoso et al., 1993). To make understanding NF-κB function more difficult, certain dimer combinations, for example RelB:RelB, have yet to be identified within cells (Hoffman et al., 2003; Marienfeld et al., 2003). Understanding how NF-κB functions within all cells, however, is greatly complicated by this very fact that NF-κB can take a number of different forms, each comprising differing combinations of NF-κB subunits being able to determine the specificity of NF-κB transcriptional activation: indeed, all having distinct, non-overlapping functions (Perkins et al., 1992; Gerondakis et al., 1999; Hoffman and Baltimore, 2006; Perkins and Gilmore, 2006).

In addition to the variety of homo- and heterodimers formed, NF-κB proteins also undergo post translational modifications such as phosphorylation (reviewed in Schmitz et al., 2001; Campbell and Perkins, 2004), acetylation (Chen et al., 2002), S-nitrosylation (Reynaert et al., 2004) and can recruit a range of other regulatory proteins and transcription factors to the enhancer/repressor region (reviewed in Perkins, 2007). Together with the inherent architecture of the enhancer/promoter itself, a number of opportunities therefore arise for highly specific gene expression in response to a given stimulus. Since this subject has been comprehensively reviewed elsewhere (Perkins, 2007) it will not be covered here.

## NF-κB activation

In the majority of non-activated cell types, NF-κB is generally retained within the cytoplasm in a transcriptionally inert form, bound to its inhibitor protein, IκB, of which there are several isoforms (IκBα, IκBß, IκBε, Bcl3 and IκBζ; reviewed in greater depth in Perkins, 2007) although basal nucleocytoplasmic shuttling of NF-κB complexes does occur. Indeed, the crystal structures of IκBα bound to truncated RelA:p50 heterodimers demonstrate that the C-terminal IκBα fragments used in those studies occluded the DNA-binding cleft of RelA through an allosteric mechanism. In the RelA:p50 heterodimer, however, only the RelA nuclear localisation sequence (NLS) is masked upon binding to IκBα: that of p50 remains unbound. Moreover, IκBα itself also contains a potent nuclear export sequence (NES). The corollary to this, therefore, is that a dynamic interplay between the NES of IκBα and the NLS of p50 appears to serve as the mechanism by which shuttling of NF-κB complexes between the cytosol and nucleus occurs (Huxford et al., 1998; Jacobs and Harrison, 1998; Johnson et al., 1999; Huang et al., 2000).

NF-κB can be activated within the cell in at least two ways (Fig. 2). The first, canonical pathway, induced by stimulation with inflammatory factors such as TNFα, IL-1ß and LPS causes phosphorylation of the IκB kinase (IKK; a complex of α, ß and two γ subunits), which in turn, phosphorylates IκB proteins at two highly conserved serine residues (Ser 32 and 26 in IκBα), leading to IκB ubiquitination and degradation by the 26S proteosome. This then releases particular NF-κB subunits, for example, RelA:p50 heterodimers, and unmasks nuclear localisation sequences in those subunits, which in turn facilitate subunit translocation to the nucleus. Once in the nucleus, gene expression is modulated by binding to specific sequences in the promoter regions of many genes including those involved in immune and inflammatory responses. It is this canonical pathway which has received all the research attention in the field of myometrial biology due to its intimate association with the activation of COX-2 (and ultimately prostaglandin) synthesis.

The second, non-canonical, pathway of NF-κB activation, which can also be induced by CD40, Lymphotoxin-ß (LT-ß), B-cell Activating Factor (BAFF) and LPS, utilises IKKα-induced phosphorylation and processing of p100 to p52 (Senftleben et al., 2001; Pomerantz and Baltimore, 2002). This results in the generation of different NF-κB heterodimers, mainly p52:RelB, which transactivate a different subset of genes compared to p50:RelA and p52:RelA (Senftleben et al., 2001; Pomerantz and Baltimore, 2002). Such gene targets are less well described but include proteins including chemokines BCL (B-Lymphocyte Chemokine), ECL (Epstein Barr Virus-induced-1 ligand chemokine), SDF-1 (Stromal Cell-Derived Factor-1) and SLC (Secondary Lymphoid Organ Chemokine) (Bonizzi et al., 2004). The function, if any, for this pathway or these gene products in the myometrium, however, is presently unclear.

There are also other mechanisms by which NF-κB can be activated in cells. For example, Imbert et al. (1996) described the phosphorylation of IκB at Tyrosine-42 in response to hypoxic insult. This modification of IκBα was reported in Jurkat cells but did not induce IκBα degradation seen for other signals such as TNFα. Instead, phosphorylation of Tyr-42 induced direct dissociation of IκBα from NF-κB: the possible relevance to parturition research, however, currently remains unclear. Induction of phosphorylation at Tyr42, possibly by casein kinase II (Schoonbroodt et al., 2000) or by tyrosine kinase activity (Mukhopadhyay et al., 2000) may also induce IκB degradation and subsequent NF-κB activation. Moreover, both U.V. stimulation and activation of the Her2 oncogene can result in casein kinase II-induced phosphorylation of IκBα in a C-terminal region (the PEST domain) distinct from that utilised by the IKK complex (reviewed in Perkins 2007). It is unclear whether such atypical NF-κB activation mechanisms such as U.V. or oncogenic stimulation, could function during parturition: hypoxia-induced IκBα dissociation, however, could be envisaged to play a role and will be discussed.

### NF-κB DNA-binding

The consensus NF-κB binding site is generally viewed as 5'-GGGRNYYYCC-3' (where R = A or G; N = A, C, T or G and Y = C or T) although there are many functional variations of this (Chen and Ghosh, 1999; Natoli et al., 2005). It is also estimated that there are in excess of 3000 κB sites within the human genome (Natoli et al., 2005). Importantly, other mechanisms of regulation exist, for example, NF-κB dimer exchange on a given promoter over time can regulate the level of expression from that gene (Saccani et al., 2003). Furthermore, single base changes in a κB site may influence the degree to which promoter DNA is distorted upon NF-κB binding (Leung et al., 2004). This, in turn, can subsequently affect which cofactors are recruited to the promoter by that particular DNA-bound NF-κB subunit (Perkins, 2007). Consequently, the inherent architecture of a given NF-κB-responsive enhancer/promoter *in vivo*, can itself directly influence the level of gene expression through its ability to selectively recruit different NF-κB hetero- or homo-dimers in response to a

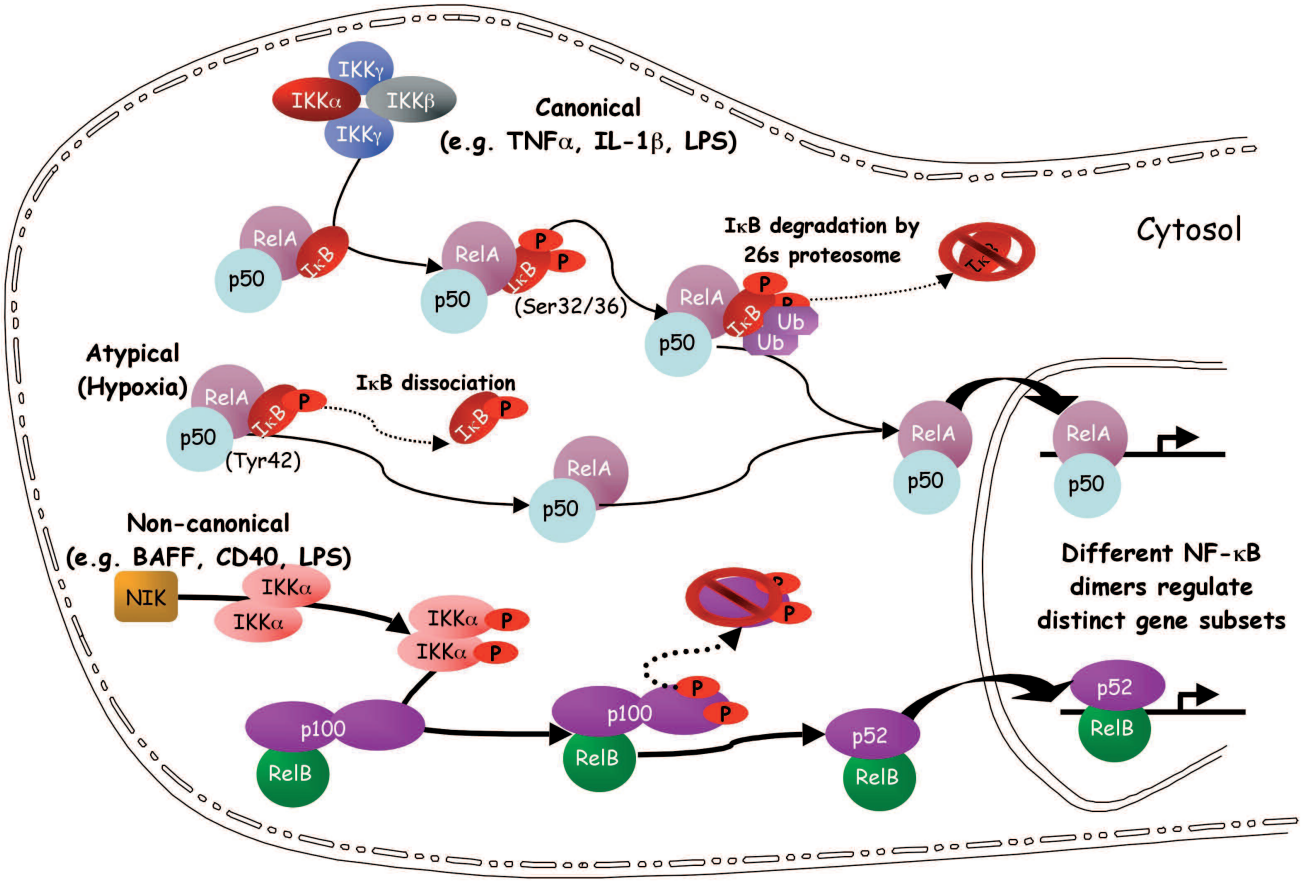


**Fig. 2.** Schematic of the NF-κB activation pathways. In the canonical activation pathway (top figures), ligands, such as tumour necrosis factor-α (TNF-α) engage cell surface receptors and induces a kinase cascade that phosphorylates the IκB kinase (IKK)α and ß subunits. Once activated, IKK then phosphorylates IκB, which, in turn, is ubiquitinated and degraded by the 26S proteosome. RelA:p50NF-κB is then free to translocate to the nucleus where it can activate gene expression. Atypical activation of NF-κB can also be induced through cellular stress, in particular, through hypoxia. In this example, (middle figures), hypoxia induces phosphorylation of IκBα at Tyr-42 thereby stimulating dissociation of the NF-κB:IκBα complex freeing NF-κB to induce gene expression: the role of this pathway in the myometrium remains undefined. In the non-canonical activation pathway (lower figures), agonists such as B-Cell Activating Factor (BAFF) or lymphotoxin-ß (LT-ß), engage the cell surface receptor and activate the NF-κB -inducing kinase (NIK). The p100 NF-κB subunit, which resides in the cytoplasm, and can functionally serve as an IκB-like protein maintaining RelB in the cytosol, is then phosphorylated by NIK. Once phosphorylated, p100 is also ubiquitinated which induces proteolysis and removal of the IκB-like p100 C-terminal domain. This generates RelB:p52 NF-κB subunits which enter the nucleus and modulate a different subset of genes to those regulated by RelA:p50 NF-κB . There is a degree of cross-talk between each of the activation pathways which is not highlighted here. Other factors that effect the fine tuning of NF-κB -mediated gene regulation are not illustrated here. Figure 2 is based upon an original artwork published in Chapman et al. (2004); Copyright 2004 The Endocrine Society; with permission.

given stimulus (reviewed in Perkins, 1997; Hoffman et al., 2003; Hoffman and Baltimore, 2006).

## Cross-Talk between NF-κB and other Uterine Pathways

NF-κB plays a pivotal role in governing cellular processes of all tissues and organ systems within the body: the uterus being no exception. Indeed, a role for NF-κB in regulating implantation, the menstrual cycle and parturition have all been reported by a number of authors (see Chapman et al., 2002 and references therein). It should be made clear to the reader, however, that NF-κB, whilst serving a pivotal role within such cellular signalling processes, does not function in isolation from other intra-cellular signalling pathways. Each cell is exposed to a continually changing extracellular milieu of stimuli which will activate numerous signalling messengers. Consequently, we will briefly examine mechanisms that contribute to the ability of NF-κB to govern gene expression. This first is through the employment and utilisation of different NF-κB dimer combinations and secondly also that of cross-talk with the cAMP/PKA pathway.

### The role of NF-κB subunits in the myometrium

The role of NF-κB in human parturition has generally focused on regulation of the inflammatory pathway in various reproductive tissues. Whilst such studies provide vital information about the regulation of individual genes associated with labour, they concentrate on a limited number of characterised promoters, for example COX-2, which while important does not act in isolation. Moreover, in the context of myometrial biology, there is little data regarding the spatio-temporal expression and activity of other NF-κB subunits in this smooth muscle during pregnancy and labour. Since the myometrium is exposed to a complex milieu of cytokines, chemokines and other signalling factors, one could easily envisage that other NF-κB subunits are involved in myometrial smooth muscle gene expression.

Chapman et al. (2004) did examine the temporal expression and activity of each NF-κB subunit in homogenates of human myometrial biopsies. This study demonstrated that whilst total protein levels of RelA, p50 and c-Rel decline in labour, there is a change in dimer configuration between non-pregnant tissues (mostly p50 dimers; potentially transcriptionally inert) and labouring extracts (RelA:p50 dimers; transcriptionally active).Of interest was the fact that while p100 subunits were detected in their experiments on whole human myometrial homogenates from non-pregnant, pregnant or labouring subjects, the processed form, p52 subunits, were not observed. The reason for this, however, remains unclear: the obvious caveat of that data is the use of crude muscle biopsies from a single time period, which may have easily missed the activation of the non-canonical pathway in such studies.

It is also prudent to consider the non-canonical mode of NF-κB activation as it is now well documented that p52:RelB dimers formed through this non-canonical mechanism can maintain prolonged expression of RelA:p50 induced genes (Saccani et al., 2003) but also harbour the ability to activate very different, albeit smaller, repertoires of genes: indeed, Fusco et al. (2009) suggest that κB site selection by the p52:RelB dimer utilises A:T-rich middle segments compared to conventional p50:RelA heterodimers, thereby permitting it to bind to a much broader array of κB sites within different target genes (Fusco et al., 2009). If this is the case in human myometrium then as a field, we are overlooking a further level of complexity of how the NF-κB family governs gene expression in this organ.

It is also noteworthy that virtually all studies of NF-κB function in the reproductive tract rarely consider the persistence of NF-κB promoter occupancy. Once the initial stimulus has occurred, the longer-term effects are not studied. This important point must be appreciated in the case of myometrial contractility, as it is likely that such NF-κB-sensitive genes must remain activated at the very least until parturition has ceased and possibly even until the uterus has completed involution. The corollary to this is that one must ask what keeps NF-κB regulated genes activated in the myometrium over a period of hours.

At present, there is no data from the myometrium to determine how such prolonged gene expression occurs. Instead, we are left to extrapolate from other cell lines. Using the murine macrophage line RAW264.7, Saccani et al. (2003) demonstrated that NF-κB RelA was activated and bound to various promoters in a wave-like fashion. Rapid promoter occupancy being seen by RelA on IκBα, macrophage inhibitory protein-2 (MIP-2) and manganese superoxide dismutase (MnSOD) genes, whilst slower kinetics of occupancy were observed for macrophage chemo-attractant protein-1 (MCP-1), RANTES and IL-6. Activation of MnSOD and MCP-1 by RelA persisted in spite of IκBα resynthesis: those authors suggesting that IκBα could possibly prevent removal of the DNA-bound NF-κB complex from the promoter by IκBα (Saccani et al., 2003).

Moreover, Saccani et al. (2003) also demonstrated that rapidly activated dimers such as RelA:p50 were slowly replaced with different dimer combinations including RelB:p52 on certain promoters over a period of time to ensure that expression was either maintained from those promoters at a level consistent with the needs of the cell or, alternatively, shut down completely. Whilst it is tempting to speculate that similar processes may be occurring in human gestational tissues during parturition, there is scant experimental data to support this hypothesis at present.

The IκB family of proteins also tightly regulate NF-κB function. Interestingly, using electrophoretic mobility shift assays (EMSAs), Allport et al. (2001) observed prolonged NF-κB DNA binding in the human amnion, and it was later suggested that elevated levels of IκB2

were responsible for prolonging this effect. It should be clarified, however, that total DNA binding by EMSA, an *in vitro* method, is a poor reflection on the specific NF-κB activity at each individual promoter within its natural chromosomal context. Furthermore, Huang and Miyamoto (2001) reported that IκB, would prevent nuclear export of NF-κB as well as repressing NF-κB DNA binding activity (Huang and Miyamoto, 2001). These data from the amnion suggest a prolonged response prior to delivery could be needed to ensure sufficient inflammatory mediators and tissue remodelling enzymes are present for membrane rupture. The post-parturition data are less clear: the amnion is a decidual tissue, shed from the uterus once the foetus has been delivered and as stated above, it is difficult to correlate EMSA data to specific NF-κB activity on a given promoter.

It is also noteworthy that persistent TNFα stimulation of fibroblast cells actually prevented the complete re-synthesis of IκBα and induced a prolonged nuclear localisation of NF-κB (Poppers et al., 2000). In light of the fact that TNFα and many other proinflammatory mediators are present within the myometrium and decidua at term and that parturition itself is a drawn out event, it is likely that prolonged activation of NF-κB within those tissues will be a central requirement: the mechanism by which that occurs, however, has yet to be resolved.

### A role in the myometrium for NF-κB activated by other Mechanisms

As stated above, it is unclear whether atypical NF-κB activation through a hypoxia-induced mechanism functions during human parturition. Clearly, the myometrium will experience hypoxic episodes during this process. Indeed, during foetal expulsion, the strength of myometrial contraction can be so great as to occlude local blood flow to that tissue: even on such a temporary basis, repeated induction of hypoxia can have significant detrimental effects upon the force of myometrial contractility (Wray, 1990; reviewed in Taggart and Wray 1998; Bugg et al., 2006). For example, under hypoxic conditions, the force of contraction in isolated whole myometrial biopsies is seen to decrease (Bugg et al., 2006). Moreover, similar observations have been made in the presence of reactive oxygen species and peroxide ($H_2O_2$; Warren et al., 2005). Interestingly, and as stated above, such toxic insults have been demonstrated to rapidly induce NF-κB activation in experimental cell lines (Imbert et al., 1996; Mukhopadhyay et al., 2000; Schoonbroodt et al., 2000).

During a hypoxic insult, the cell normally responds through activation of hypoxia inducing factor (HIF) families of transcription factors (Rocha, 2007). Such transcription factors then coordinate the appropriate protective response which includes transcription of genes involved with oxygen supply (erythropoietin, haem-oxygenase-1, VEGF-A), a variety of metabolic enzymes including phosphofructokinase aldolase-A and glucose transporters, and factors governing cell growth (IGFBP-1) and cell death (Rocha, 2007 and references therein). Modulation of mRNA translation also occurs as part of this response to ensure that restoration of normal oxygen homeostasis occurs as rapidly as possible.

Interestingly, induction of hypoxia has also been shown to induce activation of NF-κB: HIF-1α having an active κB motif within its promoter region (Van Uden et al., 2008). Furthermore, this study also demonstrated that TNFα, a potent inducer of NF-κB, could also activate HIF-1α expression. Consequently, in the context of myometrial biology, one could envisage a mechanism where such inflammatory mediators present within the myometrium at term also offers protection to that organ during periods of contraction-induced ischemia: experimental data to support this notion, however, is lacking at the present time.

Obviously, in terms of developing novel tocolytic agents targeted against NF-κB function, failure to appreciate the potential of the hypoxia-induced NF-κB activation pathway could result in development of agents which, whilst able to induce tocolysis, also abrogate the protective mechanisms against hypoxia: this in itself could then induce its own series of unwanted medical and surgical sequelæ.

### The myometrial Gαs/cAMP/PKA pathway

One of the most significant physiological adaptations of the uterus to pregnancy is the development of a relative state of myometrial smooth muscle inactivity termed quiescence. At the onset of labour the state of myometrial quiescence ends and a series of powerful uterine contractions act to expel the infant. There is growing evidence indicating that components of the cyclic AMP (cAMP) signalling pathway are differentially expressed in the human myometrium during pregnancy thereby potentiating the maintenance of uterine quiescence until term. These include calcitonin gene related peptide (CGRP) receptors (Dong et al., 1999), chorionic gonadotrophin/LH receptors (Zuo et al., 1994) and the adenylyl cyclase stimulatory G-protein Gαs, whose expression levels are considerably increased within the myometrium during gestation causing an increased production of cAMP and activation of protein kinase A (PKA); these factors are subsequently reduced in labour (Europe-Finner et al., 1993, 1994, 1996; Phaneuf et al., 1993; Bailey et al., 2000, 2002; MacDougall et al., 2003; Phillips et al., 2002, 2005). The mechanism by which down-regulation of Gαs expression occurs, however, remains unclear, although recently it was reported that the Gαs promoter was regulated by Sp-like transcription factors requiring phosphorylation by PKA (Phillips et al., 2005) and that induction of NF-κB by pro-inflammatory mediators could repress expression from a synthetic Gαs promoter construct (Chapman et al., 2005).

In many other systems within the body there is

considerable cross-talk between various G-Protein Coupled Receptors (GPRC) and NF-κB [reviewed in Ye, 2001 and Fraser, 2008]. For example, with regard to Gαs and cAMP signalling, it has been well documented that elevation of cAMP and activation of PKA have been shown to inhibit NF-κB-mediated transcription in a number of non-myometrial cell lines (Chen and Rothenberg, 1994; Neumann et al., 1995; Parry and Mackman, 1997). This thesis, however, is further complicated by reports detailing an association between RelA and PKA (Zhong et al., 1997), with PKA being required to activate NF-κB DNA-binding in a cAMP-independent fashion (Zhong et al., 1998). Importantly, Chapman et al. (2004) identified an association between RelA and the catalytic subunit of PKA in pregnant myometrial homogenates suggesting there may be cross-talk between NF-κB and the Gαs/cAMP/PKA pathways within the myometrium (Chapman et al., 2004). As above, PKAcα (the catalytic subunit) is generally viewed as being held in an inactive complex by associating with two regulatory subunits. The fact some myometrial PKAcα is sequestered into an inactive complex is of interest. RelA-associated PKAcα is not bound to the normal regulatory subunits but is, instead, bound by IκBα which serves a similar regulatory function over PKAcα, maintaining it in an inactive state until the cell is exposed to a RelA-inducing stimuli (e. g. IL1-ß or TNFα; Zhong et al., 1997; 1998). Therefore, it has been suggested that during quiescence, a distinct pool of RelA is associated with IκBα-regulated, cAMP-independent PKAcα. Upon induction of labour, however, the regular PKA pathway is repressed (Chapman et al., 2004). RelA, however, could still undergo rapid PKAcα-mediated phosphorylation in response to the appropriate stimuli to then up-regulate various contractile associated proteins, such as COX-2 or myosin light chain kinase (MLCK) which would propagate pro-labour signals and contraction of myometrial myocytes. Consequently, it was of great significance that both the pro-inflammatory factor, TNFα and the RelA NF-κB subunit could repress the promoter of the pro-quiescent gene product, Gαs (Chapman et al., 2005).

The mechanism by which RelA induced repression of Gαs was not through direct DNA binding by NF-κB but was documented to be mediated through competition

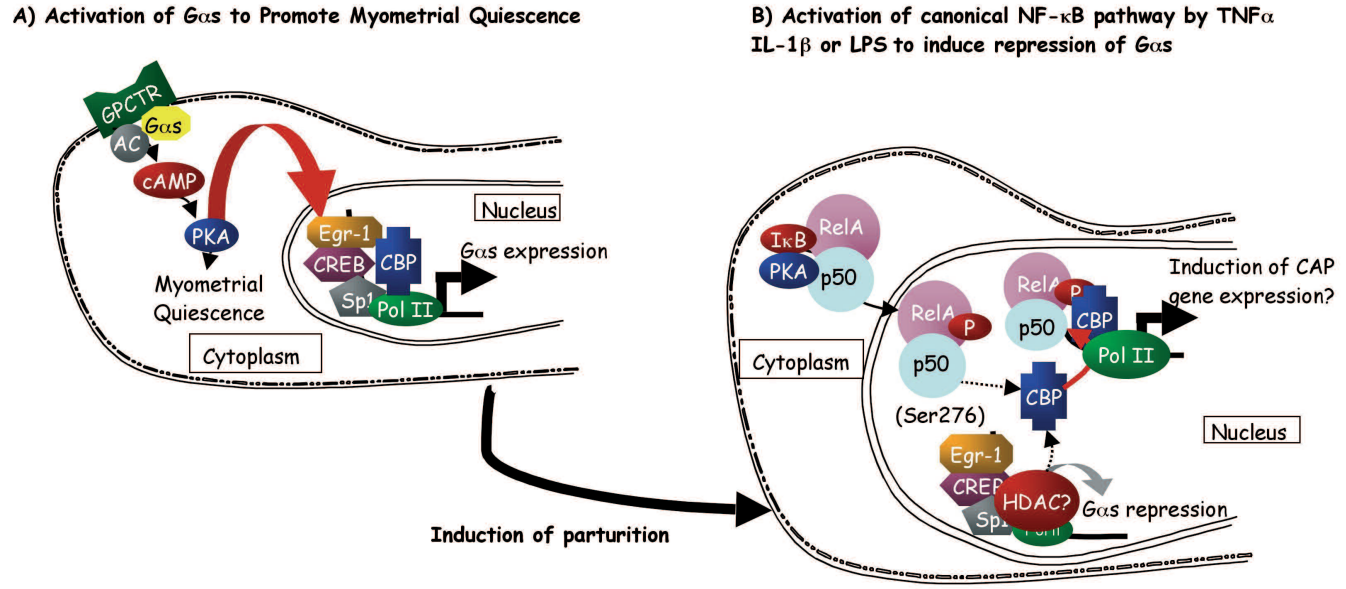


**Fig. 3.** Schematic to illustrate how relA NF-κB and CBP may repress the Gαs gene. During pregnancy engagement of G-protein-coupled trans-membrane receptor (GPCTR) activates the Gαs/adenylyl cyclase (AC) pathway which causes an increase in intra-cellular cyclic adenosine monophosphate (cAMP) and activation of protein kinase A (PKA). PKA is then able to phosphorylate a number of nuclear proteins, including cAMP response element binding protein (CREB) which, in turn, serves as a binding partner for CREB binding protein (CBP). Once CBP is associated with CREB at a given promoter, in this case Gαs, it would serve to both acetylate histones within this region, thus facilitating promoter melting and also ensure all the necessary transcription factors for Gαs expression are in the correct spatial positions thereby ensuring the promoter can be transcribed by RNA polymerase II (Pol II). Activation of NF-κB at or prior to parturition by pro-inflammatory mediators, such as TNFα (acting through the TNFα receptor; TNFαR) or bacterial LPS (binding to Toll-like Receptor 4; TLR4) stimulates the phosphorylation and nuclear localization of RelA. Once inside the nucleus, RelA binds to specific target genes, for example COX-2, where it is then able to recruit CBP to the promoter region. Since there is only a finite amount of CBP, a more favorable interaction with RelA on the COX-2 promoter over CREB may result in the loss of CBP from the Gαs regulatory region. Loss of CBP would then be expected to down-regulate the Gαs gene, possibly through the replacement of CBP with a repressor protein/complex such as a histone deacetylase (HDAC). Figure 3 is based upon an original artwork published in Chapman et al. (2005); Copyright 2004 The Endocrine Society; with permission.

for limiting cellular amounts of the co-activator and potent histone acetyltransferase CREB-binding protein (CBP) (Chapman et al., 2005; see Figure 3). A number of groups have examined the levels of CBP and other co-factors including steroid receptor co-activator (SRC) family of proteins in myometrium at term and labour and found all co-activators to be decreased during parturition (Condon et al., 2003; Long et al., 2005). Clearly a loss of such co-activators would increase the intra-cellular competition for CBP/p300 or analogous HAT-containing proteins implying that only a reduced number of promoters would have access to such HATs. Consistent with this notion is evidence that documents similar molecular circuits which serve to regulate the p53-mediated apoptotic pathway (Wadgaonkar et al., 1999; Webster and Perkins, 1999), synergism between CBP and SRC-1 (Smith et al., 1996), steroid receptor-mediated repression of inflammation (Kamei et al., 1996), macrophage development controlled by the Jak/STAT and Ras/AP-1 pathways (Horvai et al., 1997; Guberman et al., 2003) and the RelA-induced repression of the CREB-responsive gene phosphoenolpyruvate carboxykinase (PEPCK) (Waltner-Law et al., 2000). The salient question, however, remains: what cellular event determines which promoter receives such factors? Is it the particular cellular context at that given moment in time: essentially a stochastic mechanism or are events more tightly regulated? These are key questions whose answers are needed if we are to understand how the human myometrium switches from a state of prolonged quiescence to one of short-term contractility.

### Cross-talk with other GPCR?

Here we have briefly described earlier work demonstrating cross-talk between NF-κB and Gαs signalling in the myometrium. Clearly, there is significant published evidence to demonstrate that other GPCR activate NF-κB signalling too (see Ye, 2001 and Fraser, 2008). There is, however, a lack of data defining how such GPCR are regulated at the level of the gene. One study has demonstrated that NF-κB is required to stimulate expression of the Gαi2 gene (Arinze and Kawai, 2005). Other groups, meanwhile, have provided indirect experimental evidence for the involvement of NF-κB in governing expression of Gαi and Gαq in human airway smooth muscle (Hotta et al., 1999). Clearly, signalling through both Gαi and Gαq will activate NF-κB (Ye, 2001 and Fraser, 2008) although this will be dependant on the cognate receptor that the protein is coupled to: for example, IL-8 expression is mediated through Gαi (Ye, 2001), while both oxytocin and prostaglandins E and F function through Gαq to induce myometrial contraction (reviewed in Smith, 2007).

### Conclusions

It is clear that NF-κB plays a pivotal role in governing cell function in all systems throughout the body: the myometrium being no exception. What remains uncertain, however, is the depth to which NF-κB is involved. Obviously, it is a key regulator of many pro-inflammatory agents that have been recorded as being elevated in reproductive tissues at term but it is less clear whether this is a cause or effect of the parturition process. In humans, of course, the corollary is that we are also limited in our understanding because of the limited nature of the studies we can perform: our models must be chosen carefully to reflect this.

Despite this, it remains unequivocal that understanding how NF-κB governs myometrial function is vital if there is to be any progress in developing more effective tocolytics with which to prevent premature birth. Due to the wide range of cellular processes regulated by NF-κB, however, a total blockade of its action would be undesirable. Consequently, identifying which gene networks are activated or repressed by this protein family would greatly assist in identifying pharmacological compounds able to cause a more specific modulation of its function. Consequently, in order to identify new NF-κB-regulated genes, high throughput sequencing of whole genomes from reproductive tissues including myometrium, is one possible experimental strategy that must be fully considered if we are to understand how this tissue is governed by NF-κB.

Focussed large scale screens of NF-κB binding site location across chromosome 22 have already provided a wealth of new information regarding how NF-κB may modulate gene expression: indeed Martone et al. (2003) observed that while many NF-κB sites were enriched in 5' regions of the genome, many sites were also identified within intronic regions of chromatin. Moreover, binding to DNA alone was, in some cases, not sufficient to activate gene expression suggesting further layers of regulation (as detailed above) need to be overcome to ensure expression of certain genes. Consequently, such data reinforce the need to complete such studies within human myometrial tissue to ensure the data we obtain about NF-κB function is based on genes in their natural chromosomal environment, where natural promoter selectivity will be functioning in concert with cross-talk between other signalling pathways within the cell.

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