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REVIEW

Transcriptional control of parturition: insights from gene regulation studies in the myometrium

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ABSTRACT: The onset of labour is a culmination of a series of highly coordinated and preparatory physiological events that take place throughout the gestational period. In order to produce the associated contractions needed for foetal delivery, smooth muscle cells in the muscular layer of the uterus (i.e. myometrium) undergo a transition from quiescent to contractile phenotypes. Here, we present the current understanding of the roles transcription factors play in critical labour-associated gene expression changes as part of the molecular mechanistic basis for this transition. Consideration is given to both transcription factors that have been well-studied in a myometrial context, i.e. activator protein 1, progesterone receptors, oestrogen receptors, and nuclear factor kappa B, as well as additional transcription factors whose gestational event-driving contributions have been demonstrated more recently. These transcription factors may form pregnancy- and labour-associated transcriptional regulatory networks in the myometrium to modulate the timing of labour onset. A more thorough understanding of the transcription factor-mediated, labour-promoting regulatory pathways holds promise for the development of new therapeutic treatments that can be used for the prevention of preterm labour in at-risk women.

Key word: myometrium / gene regulation / transcriptional regulatory network / transcription factors / pregnancy / labour / contractile / smooth muscle cells

Introduction

Labour is a remarkably complex event orchestrated by physiological processes that work together to control the timing of birth. In the months preceding this event, the uterus, a vital myogenic organ in the female reproductive system, is the epicentre of critical labour-preparatory processes. The myometrium, a uterine muscle layer mainly composed of smooth muscle cells (SMCs), maintains a state of quiescence during pregnancy. Labour is initiated when contraction-associated genes in SMCs are activated in response to a combination of hormonal, inflammatory and mechanical signals (Shynlova *et al.*, 2009, 2013a). Once these contraction-associated genes are sufficiently expressed, myometrial cells develop the ability to synchronously produce contractions strong enough to expel the foetus from the uterus. The transition of SMCs from quiescence to contractility typically occurs at term, or the final stage of pregnancy, when the foetus is developmentally ready for birth.

In some cases, however, contractions commence prior to term, causing women to go into labour too early. Risk factors ranging from pre-existing health conditions and genetic pre-disposition to bacterial or viral infections contracted during the gestational period can trigger the premature transcription of contraction-associated genes in SMCs and consequent initiation of myometrial contractions in advance of term. In such instances, the mother undergoes preterm labour (PTL), i.e. labour that occurs prior to 37 weeks of gestation. As leading cause of increased neonatal mortality and morbidity worldwide, PTL often results in the birth of a stillborn or underdeveloped infant. The health consequences for preterm babies are not limited to the neonatal period but extend throughout their lives, placing a significant financial burden on families, caregivers and the economy (Jehan *et al.*, 2009; Johnston *et al.*, 2014; Shah *et al.*, 2018; Chawanpaiboon *et al.*, 2019). The most prominent subtype of PTL, is spontaneous PTL which occurs without any known cause and accounts for the vast majority (70%) of PTL cases (Goldenberg *et al.*, 2008). The few preventative antibiotic or uterine tocolytic-based options available for women at risk for PTL can only prolong pregnancy for up to 7 days and/or can pose adverse risks for the premature infant (Abou-Ghannam *et al.*,

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2012; Haas et al., 2012). In the absence of effective treatment options for PTL, new approaches are warranted. Their design would be greatly enhanced by a more thorough knowledge of myometrial biology and the mechanisms underlying the myometrial transition to the contractile state.

Here, we focus on the critical role transcriptional regulatory networks play in SMCs to maintain uterine quiescence during pregnancy and promote contractility at labour onset. Whereas prior reviews on this topic have focussed largely on post-transcriptional cellular events, we concentrate on recent research advances that have begun to shed light on the gene regulation mechanisms at the genomic and primary transcriptomic levels. We highlight the crucial role transcription factors play in defining gestation stage-related myometrial tissue phenotypes. By identifying a comprehensive set of key molecular players in the myometrium that are involved in the onset of labour-associated contractions, this research can be employed in the development of efficient drugs to prevent preterm birth.

Physiology of labour

From a physiological standpoint, the myometrium spans two regions in the human uterus: the lower uterine segment (LUS) and the upper uterine segment, i.e. fundus (FUN). Both uterine regions adopt differing phenotypes throughout gestation. During pregnancy, the foetus occupies a relaxed FUN, which prevents the application of pressure on the cervix. The LUS, on the other hand, displays an increase in muscular tone at this time to support foetal positioning in the FUN. At term, however, the two regions undergo a state reversal. Increased activity of the FUN ensures the initiation and continuation of forceful labour contractions to expel the foetus, while the relaxation of the LUS allows for the passage of the foetus through the birth canal (Challis JRG et al., 2000; Liu et al., 2015; Li et al., 2016, 2018). Unless otherwise stated in this review, we will describe the activity of contractile myometrial cells associated with labour, and, for the sake of clarity, refer to the myometrial state as generally quiescent during pregnancy and contractile during labour.

To perform its dynamic roles in sustaining pregnancy or bringing about labour, the myometrium undergoes extensive remodelling, with simultaneous intra- and extra-cellular compositional changes unfolding throughout gestation. For the majority of the pregnancy period, the myometrium maintains a state of quiescence, one primarily facilitated by the endocrine hormones, progesterone and relaxin, as well as parathyroid hormone-related protein and nitric oxide (Challis and Lye, 1994). Near the end of term, a combination of mechanical stretch- and endocrine agonist-driven signals begins to be transmitted. Mechanical stretch occurs in response to applied tension on the uterine wall by the growing foetus. Concurrently, foetal-stimulated cortisol is released via the hypothalamus–pituitary–adrenal gland axis, a multiple organ-mediated series of feedback mechanisms that control the timing of birth. This event in turn alters the levels of other critical hormones, including progesterone, oestrogen and corticotropin-releasing hormone and further contributes to the release of uterotonic (i.e. contraction-activating) molecules like prostaglandins and oxytocin (Weiss, 2000). Acting on the uterus, these molecules incur increased cytokine synthesis in SMCs, thereby promoting an inflammatory myometrial environment. A cascade of physiological events including cervical ripening, decidua activation and foetal membrane rupture, is incited before culminating in forceful uterine contractions that allow for expulsion of the foetus and, subsequently, the placenta. After delivery, oxytocin-mediated uterine involution or the reverting of the uterus back to the non-pregnant, quiescent state, can in time allow its preparation for a potential new pregnancy (reviewed in Challis JRG et al., 2000).

Cellular biology of labour

Apart from embedded vasculature and extracellular matrix (ECM) components, the myometrium primarily consists of smooth muscle tissues. Organised into bundle fibres known as fasciculata, these tissues comprise the circular and longitudinal muscle layers of the myometrium. Individual muscle fibres contain SMCs (Shynlova et al., 2005). During the gestational period, SMCs initially exhibit an early proliferative phase before transitioning to a synthetic phase in which cells undergo hypertrophy or an increase in individual cell size (Shynlova et al., 2009). This phase is accompanied by an expansion of the ECM through increased expression of matrix proteins (Shynlova et al., 2004). Prior to the exit of the myometrium from a state of quiescence, new SMC-modifying events take place. Increased release of pro-inflammatory cytokines and chemokines from maternal uterine tissues and foetal membranes promote a process referred to as 'sterile inflammation' (Osman et al., 2003; Hamilton et al., 2012; Shynlova et al., 2013a, b). Peripheral maternal leukocytes like neutrophils and macrophages are consequently activated (Thomson et al., 1999), infiltrating the uterus and secreting uterotonins and matrix metalloproteinases (ECM protein-digesting proteolytic enzymes) within their new environment (Yellon et al., 2003; Gomez-Lopez et al., 2014). These events then bolster maternal and foetal membrane rupture, cervical ripening and remodelling of the myometrium. With the gestational period moving still closer to its end, myometrial cells begin to up-regulate contraction-associated proteins (CAPs). For instance, the protein levels of one of the most well-studied labour-associated CAPs, gap junction alpha-1 (GJA1, also known as connexin-43 (CX43)) sharply increase between term non-labouring and active labouring myometria in rodent and primate models (Garfield et al., 1979, 1982; Sakai et al., 1992; Lye et al., 1993). Sufficient GJA1 protein quantities drive the formation of gap junctions between neighbouring SMCs, facilitating cell-cell coupling and the intercellular transfer of key metabolites and ions which supports the coordination of myometrial contractions. Ensuing transient changes in plasma membrane composition allows for a sharp influx of calcium ions from the extracellular space into SMCs (reviewed in Challis JRG et al., 2000). High intracellular ion quantities then prompt an effective relay of excitatory electromechanical signals in SMCs that initiates the continual cycling of actin-myosin cross-bridges, the cellular basis of repeated, involuntary labour-associated contractions.

Epigenetic and genomic bases of labour

Since the forceful contractile activity of the myometrium depends upon the production of sufficient quantities of proteins like GJA1 (reviewed in Challis JRG et al., 2000), the question of how their expression is strongly activated near the end of the gestational period arises. The accumulation of labour-associated proteins in a relatively short window of time is reflected by a similar up-regulation trend at the level of transcription: RNA sequencing (RNA-seq) data generated from myometrial tissues in human and rodent gestational models

indicate that large-scale changes in mRNA levels occur alongside the myometrial transition from dormancy to contractility (Chan *et al.*, 2014; Migale *et al.*, 2016; Stanfield *et al.*, 2019; Shchuka *et al.*, 2020; Wu *et al.*, 2020). Further evidence for a transcriptional role in the onset of labour has been provided by total RNA-seq analyses in mouse tissues, as the majority of genes displaying an increase in mRNA levels at labour onset also exhibit a significant gain in reads at their introns, evidence of recent, active transcription events (Shchuka *et al.*, 2020). Genes that become more highly transcribed include those encoding well-studied CAPs like oxytocin receptor (OXTR), and prostaglandin-endoperoxide synthase 2 (PTGS2, also known as COX2) and again, GJA1. Indeed, the generation of a mouse model in which the *Gja1* gene was conditionally deleted in SMCs revealed significantly prolonged labour in 82% of mutant mice (Döring *et al.*, 2006), underscoring the importance of *Gja1* up-regulation from its gene template for the initiation of birth.

The specific transcriptional up-regulation of contractility-driving genes at term implies labour-associated involvement of both transcriptional activators and regions of the genome at which these factors exert their regulatory control. As studies conducted in other cell types have revealed, the process of transcription typically involves the assembly of transcription factors into large multi-factor complexes that engage with specific genomic sequences residing within chromatin, i.e. nuclear protein-enriched DNA (Moorthy *et al.*, 2017). To up-regulate the expression of a gene, transcription factors commonly bind gene promoters; additionally, in select gene contexts, distal regulatory regions located as far as even multi-megabase distances from promoter regions can be bound by factors and brought in closer physical proximity to gene promoters to amplify the up-regulation signal (Carter *et al.*, 2002; Tolhuis *et al.*, 2002; Lettice *et al.*, 2003; Sanyal *et al.*, 2012). Although transcription factor complexes and chromatin folding events that orchestrate gene regulation processes have been well-documented in some cell types, SMCs do not fall in this category. Nevertheless, existing studies do support a genomic basis for the onset of labour. *In vitro* experiments have highlighted that the *Gja1* promoter can drive more prominent luciferase reporter expression in the presence of certain combinations of activator protein 1 (AP-1) transcription factors expressed during labour (Mitchell and Lye, 2005). Later work by Nadeem et al. (2016) indicated that AP-1 proteins and progesterone receptors (PRs) co-ordinately regulate *Gja1* transcription in response to progesterone. Depending on the intracellular presence or absence of the hormone, PR isoforms localise in different sub-cellular regions and have distinct transcription factor interacting partners in pregnant and labouring human myometrial tissues (Nadeem et al., 2016). Our own recent work lends further support for the hypothesis that the regulation of labour-associated genes at term depends on access to nuclear chromatin. We observed that multiple genes up-regulated at term have a significantly greater abundance of primary transcripts during the labouring state. Furthermore, these genes contain an active histone mark signature at their promoters prior to term, but their gene bodies are more markedly associated with RNA Polymerase II (RNAPII) at term than at the start of the late gestation period (Shchuka *et al.*, 2020). Transcription from an accessible genomic template therefore plays a predominant role in the regulation of critical contractile state-driving proteins in the myometrium. Taken together, these studies build a view of the uterine transition from pregnancy to labour as one that depends on rapid and ample changes in transcriptional activity within myometrial cell nuclei (Fig. 1). Although some of the transcription factors that orchestrate these changes are known to regulate select genes, an in-depth understanding of the transcriptional regulatory network that regulates alterations in gene expression at a genome-wide level at labour onset is lacking.

Transcription factors implicated in labour-associated gene expression in the myometrium

Since the processes of pregnancy and labour depend upon the tightly controlled regulation of critical genes at a transcriptional level in the myometrium, a thorough understanding of the proteins that coordinate these regulatory events is required. Here, we review the contributions that several transcription factor families, namely AP-1 factors, hormone receptors (specifically PRs and oestrogen receptors), and nuclear factor kappa B (NF-κB) receptors, have been shown to make during the pre-contractile and/or contractile uterine states. We first outline the molecular mechanisms of these four transcription factor families, which are well-studied in a gestational myometrial context. Our final section examines the roles of additional transcription factors recently demonstrated to assist in defining pregnant and/or labouring SMC states.

Activator protein 1

AP-1 factors comprise one of the most prominently studied transcription factor families implicated in the initiation of labour-associated myometrial contractions. This group is composed of four distinct sub-families: Fos proto-oncogene (FOS), c-Jun proto-oncogene or p39 (JUN), c-Maf proto-oncogene (MAF), and activating transcription factor 1 (ATF). The structural similarity among all four factors lies in a leucine zipper region through which these factors are able to assemble into AP-1 homo- or heterodimers (Glover and Harrison, 1995). Not every sub-family member engages in the same type of dimer combination; for instance, whereas JUN proteins are capable of existing as either homo- or heterodimers, FOS proteins do not form homodimers and instead heterodimerise with JUN, ATF or MAF proteins (Nakabeppu *et al.*, 1988). Regardless of the type of dimer generated, these molecular units, once assembled, can competently bind DNA and regulate transcription of target genes.

In addition to their different dimerisation capacities, AP-1 factors display varied expression and sub-cellular localisation patterns throughout pregnancy and at the onset of term and PTL. Across the gestational period, SMCs contain relatively constant or mildly changing levels of certain AP-1 proteins, such as JUN (Nadeem *et al.*, 2018; Peng *et al.*, 2018). Contrastingly, other AP-1 proteins are significantly increased at term, including JUND (JunD Proto-Oncogene), FOS and FOSL2 (FOS Like 2, AP-1 Transcription Factor Subunit) in the human myometrium, as well as JUND, JUNB (JunB Proto-Oncogene) and FOSL2 in both mouse and rat models (Nadeem *et al.*, 2018). In rodents, the enhanced expression of these AP-1 transcription factors occurs in response to altered progesterone-driven signalling pathways and mechanical stretch signals imposed by the growing foetus (Mitchell and Lye, 2002; Oldenhof *et al.*, 2002; Mitchell *et al.*, 2004). With levels

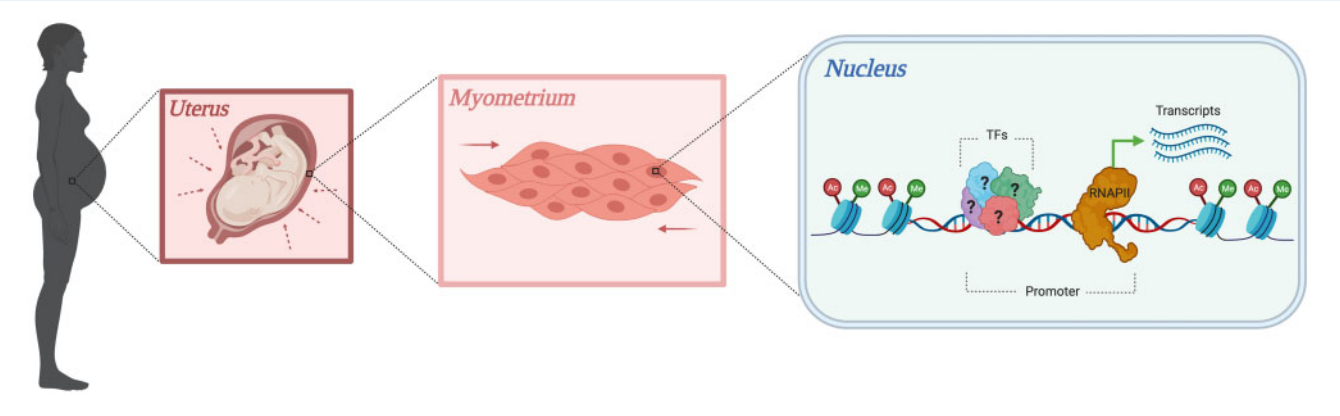

Figure 1. The onset of labour requires organism-, organ- and smooth muscle cell level changes that regulate contractility in the myometrium. The onset of labour in women depends upon the initiation of contractions in the uterus (physiological level), which are generated by labour-driving proteins in smooth muscle cells residing within myometrial tissue (cellular/molecular level). Such proteins in turn accumulate as a result of increased active transcription of labour-associated genes in the nuclei of these myometrial cells. Mediation of gene transcription involves a myriad of transcription factors as well as RNA Polymerase II (RNAPII) recruitment to contractility-driving gene promoters (epigenetic/genomic level). 'Ac' and 'Me' on nucleosomes (blue) represent Histone H3 lysine 27 acetylation (Ac, red) and Histone H3 lysine 4 methylation (Me, green) marks surrounding the transcription start site (arrow) of transcribed genes. Created with BioRender.com

markedly increased during labour, FOSL2 and JUND have been shown to localise predominantly in nuclear rather than cytoplasmic SMC fractions in both term and preterm rodent myometrium, as well as in human myometrial tissues (Nadeem *et al.*, 2018). These observations suggest a similar transcriptional role for AP-1 factors in the stimulation of SMC contractility across mammalian species and regardless of the time at which labour actually occurs (Nadeem *et al.*, 2018).

In a gestational context, AP-1 dimers are believed to regulate the expression of several labour-associated genes in the myometrium. These transcription factors bind a common core DNA motif either at a TPA response element (5′-TGAG/CTCA-3,′ or TRE) or a cAMP response element (CRE, 5′-TGACGTCA-3′ or CRE); select dimer combinations, however, preferentially bind larger variants of these motifs (as reviewed in Karin *et al.*, 1997). One or both response elements are commonly found in promoters of CAP genes (Karin, 1995; Karin *et al.*, 1997; Chinenov and Kerppola, 2001; Kumar *et al.*, 2003; Sooranna *et al.*, 2004; Terzidou *et al.*, 2005). *Gja1*, for instance, contains a strong transcription-driving proximal AP-1-binding site located closer to the TATA box and a weaker distal-binding site approximately 1 kilobase upstream of its transcription start site (Geimonen *et al.*, 1996; Echetebu *et al.*, 1999). The functional relevance of these sites has been demonstrated by experimental co-transfection of different combinations of AP-1 protein-expressing vectors and a reporter construct under the control of the endogenous *Gja1* promoter (Mitchell and Lye, 2005). This study revealed that the presence of JUN homodimers could not significantly activate reporter expression; contrarily, FOS: JUN heterodimers (specifically FOSL2: JUNB) cause increased transcription from the *Gja1* promoter (Mitchell and Lye, 2005), a finding consistent with the notion of FOS: JUN combinations acting as more potent transcriptional activators in part due to the electrostatic stability of the heterodimer (Halazonetis *et al.*, 1988; O'Shea *et al.*, 1992). Such activity, however, may not be limited to the rodent *Gja1* promoter. Besides *GJA1*, both labour-associated genes *PTGS2* and *OXTR* have promoters with AP-1 dimer-binding sites in mouse, rat and human species. At a genome-wide level in the human myometrium, Wu et al. (2020) have shown, regions of open chromatin at both promoters and intergenic regions display a significant enrichment of AP-1 motifs. Our work has furthermore shown that this AP-1 motif enrichment pattern holds true for promoters and intergenic regions associated with labour-up-regulated genes that exhibit epigenetic marks associated with transcription activation (histone H3 lysine 27 acetylation, H3K27ac and histone H3 lysine 4 trimethylation, H3K4me3) in mouse myometrial tissues both prior to and at labour onset (Shchuka *et al.*, 2020). Based on this information, it is hypothesised that JUN homodimers are bound to regulatory regions in the quiescent myometrial genome and are subsequently replaced by FOS: JUN heterodimers to activate contractility-driving gene transcription and determine the timing of labour (Shchuka *et al.*, 2020). Coupled with the observation of increased levels of nuclear FOSL2 protein during labour, these data collectively support the notion of a genome-wide transcriptional role for AP-1 factors at labour onset. Precisely where these proteins bind the genome during pregnancy and which family members are associated with the transcription of specific genes at labour onset, however, remain to be determined.

Hormone receptors

Progesterone receptors

Another set of transcription factors that contributes to the myometrial dormant and contractile phenotypes are the progesterone receptors (PRs). These nuclear receptors display altered functions in response to the steroid hormone progesterone. Two isoforms produced from the progesterone receptor (PGR) gene, progesterone receptors A (PRA) and B (PRB), have been shown to play a role in pregnancy maintenance and/or labour onset (Challis JRG *et al.*, 2000; Pieber *et al.*, 2001; Mesiano *et al.*, 2002; Merlino *et al.*, 2007; Chai *et al.*, 2012; Tan

et al., 2012; Nadeem et al., 2016, 2017). PRA and PRB are identical at the sequence level with the exception of an additional amino-terminal domain in PRB that can exhibit transactivation activity (Kastner et al., 1990; Giangrande and McDonnell, 1999). Some supporting evidence further implicates a third, smaller isoform, progesterone receptor C (PRC), in SMC activity; however, select studies have questioned whether this protein, which lacks the amino-terminal and a complete DNA-binding domain (Wei et al., 1990; Challis JRG et al., 2000), is, in fact, expressed in the myometrium (Madsen et al., 2007; Samalecos and Gellersen, 2008). Given these controversial data, we will primarily focus on highlighting the more established roles of PRA and PRB in defining SMC states, with occasional reference to findings regarding tentative PRC activity as they relate to the functions of the aforementioned two isoforms.

The structural difference between PRA and PRB sufficiently imparts contrasting transcription regulatory activities on both isoforms. Thought to facilitate uterine quiescence by repressing CAP gene transcription, PRB is expressed throughout most of the pregnancy period (Pieber et al., 2001; Mesiano et al., 2002; Merlino et al., 2007; Chai et al., 2012; Tan et al., 2012; Nadeem et al., 2017). PRB and PRC are expressed at elevated levels in the labouring FUN myometrium compared to its non-labouring counterpart and to either the labouring or non-labouring LUS (Condon et al., 2006). On the other hand, myometrial SMCs exhibit higher protein levels of PRA closer to term, which may activate genes required for contractile activity (Mesiano et al., 2002; Merlino et al., 2007; Nadeem et al., 2016). The initiation of labour has been correlated to an elevation in the ratio of PRA: PRB isoform expression in the myometrium (Mesiano et al., 2002; Merlino et al., 2007; Georgiou et al., 2016; Nadeem et al., 2016). This ratio change is thought to allow PRA to antagonise the repressive functions carried out by PRB (Merlino et al., 2007) and markedly activate the expression of contractility-driving genes.

Both the action and localisation of PR isoforms depend on the presence or absence of their ligand, progesterone. In human cells, whereas liganded PRB-mediated gene repression occurs in response to elevated amounts of intracellular progesterone, PRA-mediated activation depends on an unliganded state of this isoform (Nadeem et al., 2017). The actions of progesterone on the myometrium during gestation and the necessary removal of its inhibitory effects near term are similar across species, although some mechanistic differences exist (Csapo, 1956; Sugimoto et al., 1997; Piekorz et al., 2005). A term-restricted dramatic decrease in systemic progesterone levels in rodents (referred to as 'progesterone withdrawal') allows for the initiation of myometrial contractions (Csapo, 1956). This phenomenon, crucial for the transition to labour in most mammalian pregnancies, does not in fact occur in human and non-human primates. For the latter species, maternal systemic progesterone levels remain elevated throughout gestation and during parturition. This has led to an alternate theory of 'functional progesterone withdrawal', which proposes that human uterine cells become refractory to the progesterone signal (Csapo and Pinto-Dantas, 1965). Therefore, although the actions of progesterone are similar across species, the mechanisms surrounding the inhibition of progesterone signalling at labour onset differ across species.

The intracellular status of progesterone determines the expression levels and intracellular localisation patterns of progesterone receptor isoforms. In myometrial cells, progesterone binds PRB, which is thought to allow the now liganded receptor to translocate into the nucleus from the cytosol (Nadeem et al., 2016). Given that several studies affiliate progesterone-bound PRB with potent anti-inflammatory effects and decreased *GJA1* expression, the regulatory model for PRB action suggests that this isoform may exert its effect at AP-1-bound regions, as indicated by studies of PRB binding in breast cancer cell lines (Clarke and Graham, 2012). In a myometrial context, the PRB isoform is suspected to interact with JUN: JUN homodimers; this interaction may allow for the recruitment of a transcriptional repressor complex, p54nrb/mSin3A/HDAC, to inhibit CAP gene expression (Dong et al., 2009; Nadeem et al., 2016). On the other hand, progesterone-liganded PRA remains in the cytosol, where the receptor may regulate the synthesis and trafficking of GJA1, but is unable, at this stage, to function as a transcriptional activator (Nadeem et al., 2016, 2017). At term, PRA is up-regulated in the myometrium (Nadeem et al., 2016). At the same time, increased intracellular metabolism of progesterone by the 20α-hydroxysteroid dehydrogenase (20αHSD) enzyme frees PRA of its ligand, resulting in the translocation of this receptor into the nucleus (Nadeem et al., 2016). Reporter assays have further shown that PRA can, in the presence of FOS: JUN heterodimers, increase transcription from the *Gja1* promoter, but this activating effect is antagonised when transfected cells are treated with progesterone (Nadeem et al., 2016). At this stage, PRB localisation is restricted mainly to the cytosol, and its transcriptional regulatory capability is stifled. The prevention of its occupying the nucleus may be mediated in part by PRC, as suggested by PRC over-expression experiments in human myometrial cells (Condon et al., 2006). One study posits that PRC may sequester available progesterone hormone from PRB and/or dimerise with PRB to impede its ability to bind regions of the genome (Wei et al., 1997). These data suggest PRA works with AP-1 heterodimers to markedly elevate *Gja1* expression levels in the myometrium and thereby drive labour onset (Nadeem et al., 2016), an event perhaps enabled by antagonistic effects of PRC on PRB. Thus, the progesterone-mediating ligand states of PRB, PRA and tentatively PRC determine the intracellular location and function of these protein isoforms. Along with AP-1 factors, PRs may arbitrate whether myometrial SMCs are in a quiescent or contractile state.

As in the case of AP-1 factors, PRs likely regulate a larger repertoire of genes across the genome. Classically, these proteins bind regions containing either the consensus site or site variant of the progesterone receptor response element (PRE), 5′-GNACANNNTGTNC-3′, (where 'N' represents any nucleotide) (Chalepakis et al., 1988). However, several studies in other muscle tissues have indicated that PRs can also be active at sites bound by other DNA-binding proteins, notably AP-1 factors (Owen et al., 1998; Tseng et al., 2003; Welboren et al., 2009; Mazur et al., 2015). Genome-wide ChIP-sequencing experiments targeting total PRs have uncovered open chromatin regions occupied by the receptors in non-pregnant human myometrial tissues that differ significantly from regions in term-pregnant tissues (Wu et al., 2020). Technical experiment limitations did not allow for the ability to distinguish between PRA and PRB isoforms; however, the authors found that the regions bound by PRs strongly overlap those enriched for in AP-1 motifs. Interestingly, PR binding at intronic and intergenic regions is also comparatively higher than promoter occupancy in both non-pregnant and term pregnant tissues (Wu et al., 2020), suggesting an involvement of distal regulatory regions in gene expression control. Overall, these changes in PR occupancy across the

genome implicate PRs as crucial drivers of the transcriptional changes between pregnancy and labour.

Oestrogen receptors

Apart from PRs, the most prominent steroid hormone-regulated proteins associated with the induction of labour belong to the oestrogen receptor (ER) family. Multiple players in this family contribute to several physiological processes in the reproductive system; however, only some act as transcription factors in SMCs during the gestation period. Here, we will focus predominantly on those regulatory mechanisms mediated by oestrogen receptor alpha (ERα), the transcription factor family member demonstrated to drive labour-associated gene expression events. Encoded by the *ESR1* gene in humans, ERα becomes more strongly expressed at the onset of labour (Welsh *et al.*, 2012). In order for ERα to propel the labour-associated transcriptional program forward, the receptor depends on the presence of its ligand, oestrogen. This vital steroid hormone and its receptors affect many uterine processes throughout pregnancy, from embryo implantation to cellular events in the proliferative and contractile myometrium at different labour stages (Challis JRG *et al.*, 2000; Condon *et al.*, 2020). Throughout gestation, progesterone levels are higher than relatively low-expressed oestrogen levels, and therefore gestation is considered to be dominated more by progesterone than by oestrogen. However, with the progression of the pregnancy period, oestrogen production gradually increases and reaches peak levels at term, resulting in oestrogen dominance at this stage despite the previously discussed persisting high levels of progesterone in human pregnancies (Challis, 1971; De Hertogh *et al.*, 1975; Challis and Lye, 1994). An investigation of the gestational time point-specific activity of oestrogen through ERs is therefore essential to establish a critical understanding of the labour process.

Several studies conducted in rodent and/or human myometrial tissues have demonstrated that the oestrogen-ER connection promotes a contractile state by increasing CAP gene expression (Mesiano *et al.*, 2002). Mediated by binding with ERα, oestrogen has been shown to up-regulate CAP genes such as *GJA1* (Petrocelli and Lye, 1993; Kilarski *et al.*, 1996, 2000), *PTGS2* (Wu *et al.*, 1997; Tsuboi *et al.*, 2000) and *OXTR* (Nissenson *et al.*, 1978; Sugimoto *et al.*, 1997). To modulate the expression patterns of these genes, ERα needs to be converted from an inactive to an active form. Studies have suggested that ERs are connected to HSP90 during most of pregnancy, but that rising hormone levels partway through pregnancy can induce their release (Lee *et al.*, 2012). Oestrogen can then bind ERα at its ligand-binding domain, which allows for receptor phosphorylation, dimerisation and subsequent translocation to the nucleus (McDevitt *et al.*, 2008; Lee *et al.*, 2012; Anamthathmakula *et al.*, 2019). Through their DNA-binding domains, ERα dimers can bind genomic regions that contain oestrogen response elements (EREs) within regulatory elements for labour-associated genes. It has been proposed that, once bound to their target sites, ERα proteins recruit additional co-activator complexes and, consequently, RNAPII, thereby prompting the up-regulation of CAP genes (as reviewed in Condon *et al.*, 2020). Given that oestrogen levels rise throughout late pregnancy, it has not yet been definitively established how ERα regulates the onset of labour contractions at term in particular, rather than earlier in gestation. One theory suggests that, despite the early intracellular presence of oestrogen, SMCs may be refractory, or unresponsive, to the hormone throughout most of the gestational period. In human parturition, oestrogen signalling may be regulated by a stage-specific increase in susceptibility of ERs to the hormone as opposed to changes in oestrogen concentrations (Mesiano *et al.*, 2002). Intriguingly, Anamthathmakula et al. (2019) propose a possible mechanistic explanation through their identification of the presence of alternatively spliced isoforms of ERα in both mouse and human pregnant myometrium: ERα66 (the full-length receptor weighing 66 kDa) (Flouriot *et al.*, 2000) and ERΔ7 (a protein with the same sequence, but lacking the amino acids corresponding to exon 7 of the *ESR1* gene). During pregnancy, markedly high myometrial cellular levels of ERΔ7 were observed, which, the authors suggest, allows this isoform to heterodimerise with ERα66 to suppress the activity of the full receptor and, by extension, CAP gene expression and SMC contractility. Contrarily, at the onset of labour, ERΔ7 levels are reduced, which, according to the study's proposed molecular theory, would alleviate the aforementioned suppressive effect and allow for stimulation of an ERα66-mediated contractile response via up-regulation of CAP genes.

ERs also have an intriguing regulatory relationship with PRs with regard to the timing of labour. A study conducted by Mesiano et al. (2002) concluded that there may be a role for nuclear PRs in modulating the expression of *ESR1* after observing a positive correlation between the *ESR1* mRNA levels and the ratio of *PRA: PRB* at term. The authors further suggest that reaching the appropriate relative PR isoform ratio allows for the activation of oestrogen responsiveness in human myometrium at the end of gestation. Specifically, during pregnancy, progesterone acts through the dominant PR (PRB) to inhibit the expression of *ESR1*. When PRA levels increase, an ensuing shift in the PRA: PRB ratio leads to the increased expression of *ESR1* and stronger oestrogen responsiveness in SMCs (Mesiano *et al.*, 2002). A recent study proposes that ERs and PRs participate in the same transcriptional regulatory complex (Nadeem *et al.*, 2019). A robust interaction between PRA and ERα was observed in human term myometrium and the *Gja1* promoter was shown to be most responsive in the presence of unliganded PRA and oestrogen-liganded ERα. Furthermore, an analysis of genomic regions bound by PRs and of the open chromatin regions in the pregnant human myometrium identified a significant overrepresentation of ER motifs (Wu *et al.*, 2020). These observations suggest a genome-wide regulatory role for ER at term, one that perhaps involves cooperation with PRs in its gene activating mechanism. Although further work is required to elucidate the precise nature of potential ER-PR molecular cross-talk, it remains clear that oestrogen-mediated ER activity plays a significant role in regulating contractile gene expression programs in SMCs during pregnancy and labour.

Nuclear factor kappa B

The final set of transcription factors extensively studied in a myometrial context belongs to the nuclear factor kappa B (NF-κB) family. Sharing a 300 amino acid-long sequence known as the Rel homology domain at their amino-termini, these members include: NF-κB p65 subunit (RELA), transcription factor RELB, proto-oncogene c-Rel (c-REL), NF-κB p100/p52 subunit (NFKB2) and NF-κB p105/p50 subunit (NFKB1). The Rel homology domain mediates the dimerisation, subcellular localisation and binding of NF-κB proteins to any variation of consensus DNA sequence 5′-GGGRNYY YCC-3′ (where R is a

purine, Y is a pyrimidine, and N is any nucleotide), also known as a kB site (Chen *et al.*, 1998; Rothwarf and Karin, 1999; Chen and Greene, 2004). The binding of kB sites by their namesake factors depends in part on the activation of the NF-κB signalling pathway. The canonical pathway model holds that, in response to specific stimuli, the NF-κB inhibitor IkB protein family is targeted for degradation, which results in the release of NF-κB factors from inhibitory complexes and their entry into the nucleus.

Both the induction of a cascade of molecular events via NF-κB transcription factors, as well as its ensuing regulatory effects, hinge upon stimuli that produce a pro-inflammatory environment. Stimulation of NF-κB factors can be mediated by inflammation-associated cytokines like IL-1B (Condon *et al.*, 2006), or through the Toll-Like Receptor (TLR) system, a group of proteins that control the innate immune response of the myometrium (Lindström and Bennett, 2005; Brasier, 2006). Once activated, these factors can up-regulate a cassette of genes whose expressed products in turn mediate an inflammation-driven cytokine increase. The ensuing result is the creation of the inflammatory phenotype observed in both term (sterile inflammation) and infection-based PTL contexts (Khanjani *et al.*, 2011). Indeed, some mouse models of PTL are associated with NF-κB signalling activation. For example, PTL can be induced by lipopolysaccharide (LPS) derived from *Salmonella abortus*, which has been associated with NF-κB and AP-1 functions in the myometrium (Pirianov *et al.*, 2015). LPS leads to NF-κB signalling-mediated up-regulation of PTGS2 and labour-associated cytokines in the murine myometrium, namely CCL-2 and CXCL-1. Contrarily, other preterm birth mouse models, including those involving *Escherichia coli*-derived LPS- and PR antagonist mifepristone (RU486)-PTL induction, do not entail NF-κB activation (MacIntyre *et al.*, 2014). These studies therefore suggest that while NF-κB plays a prominent role in term labour contexts, its activity may not be as substantial in all instances where PTL occurs.

Alongside their exhibition of unequal inflammation statuses, pregnant and labouring SMCs are thought to display varying levels and/or post-translationally modified versions of different NF-κB transcription factors (Chapman *et al.*, 2004). Overall, protein from all NF-κB subunits (with the exception of RELB and NFKB2) can be detected in SMCs (Chapman *et al.*, 2004). One NF-κB factor of notable interest in several studies includes the subunit RELA. Nuclear staining in myometrium tissues collected from women in term and PTL uncovered diffuse RELA labelling associated with labour onset (Vora *et al.*, 2010). The specific stage at which RELA localises to the nucleus appears to hinge on its phosphorylation status: Khanjani et al. (2011) have shown that phosphorylated RELA, when modified on one of two key serine residues (serine-536-phospho-RELA and serine-256-phospho-RELA), is abundantly present in the nuclei of SMCs in labouring human myometrium. Interestingly, nuclear localisation of phosphorylated RELA in the upper and lower segments of the myometrium appears to be specific to labour onset, even in the absence of differential expression levels of NF-κB proteins beforehand. The findings across these studies lend some initial support to the longstanding theory that NF-κB factors mediate the transactivation of promoter regions of genes encoding the aforementioned inflammatory mediators, highlighting the particular contributions of RELA in the process.

SMC can also exhibit gestational stage-specific NF-κB factor homo- or heterodimer combinations. For instance, the expression of various NF-κB family members in the non-pregnant, pregnant non-labouring and labouring human myometrium revealed that while the levels of RELA remain constant throughout gestation and labour, the subunit composition of NFKB1 homodimers prevalent in the non-pregnant myometrium changes with the progress of gestation, since RELA: NFKB1 heterodimer formation increases s at term (Chapman *et al.*, 2004). Furthermore, non-pregnant tissues exhibit prominent levels of NFKB1 homodimers, whereas non-labouring and labouring pregnant tissues contain comparatively elevated levels of RELA-NFKB1 heterodimers (Chapman *et al.*, 2004). The authors postulate that, as in the case of AP-1 factor regulatory trends, NF-κB homodimers act as repressors and heterodimers act as stimulators of the labour-associated gene expression program (Chapman *et al.*, 2004). These results supplement the findings in Condon *et al.* (2004), which report that inhibition of NF-κB in mice delays labour at term. Collectively, these studies demonstrate that NF-κB factors consist of a complex group of proteins that, when assembled in certain combinations, can drive forward the expression program for various pro-inflammatory genes in the myometrium.

The activating capacity of NF-κB transcription factors has been posited to depend not only upon interactions among members of their own signalling pathways, but also upon other transcription factors. The regulation of NF-κB has been associated with the progesterone/PR signalling pathway through multiple molecular mechanisms, where members of the latter pathway have been shown to: induce the expression of an NF-κB inhibitor, kappa light polypeptide gene enhancer in B cells inhibitor alpha (IκBα) (Hardy *et al.*, 2006); block the proteosomal degradation of IκBα (Renthal et al., 2015); drive the expression of dual-specificity phosphatase 1 (DUSP1), which reduces the phosphorylation and subsequent nuclear localisation of RELA in breast cancer cells (Chen *et al.*, 2011); and partake in physical interactions with NF-κB that may block its transcriptional activities (Kalkhoven *et al.*, 1996; Renthal et al., 2015) (summarised in Wu and DeMayo, 2017). Additionally, PRs have been shown to have a repressive effect on NF-κB-dependent transcription in amnion cells (Allport *et al.*, 2001). Along similar lines, the treatment of immortalised human fundal myometrial cells with progesterone is able to induce the expression of *IkBa*, which can in turn block the ability of NF-κB to bind the promoter and activate the expression of the labour-associated gene *PTGS2* (Hardy *et al.*, 2006). Furthermore, treatment of pregnant mice with an anti-inflammatory cyclopentenone prostaglandin has the ability to inhibit NF-κB and delay the onset of infection-induced PTL in animal models (Pirianov *et al.*, 2015). Based on these findings, it appears that progesterone acts throughout pregnancy to either prevent or counteract the effects of NF-κB signalling, which require the activation of pro-inflammatory signals.

Approaching the onset of labour, NF-κB factors adopt a gene-activating function in the presence of late gestation- and term- up-regulated transcription factors. AP-1 factors are one set of putative interaction partners for NF-κB proteins. Peng et al. (2018), for instance, have found a positive correlation between the levels of RELA subunit and cJUN in human myometrial cells, with higher quantities of and nuclear occupancy by both factors in the late pregnant cohorts relative to their early pregnant counterparts. From a genomic perspective, Wu *et al.* (2020) have shown a significant enrichment of NF-κB motifs in PR-bound regions and, more broadly, across open chromatin regions in term pregnant human myometrium. Similarly, our work has uncovered a co-enrichment of AP-1 and RELA motifs in promoters of

genes up-regulated at the onset of labour in mouse myometrium (Shchuka et al., 2020). Both these studies imply that NF-κB adopts a genome-wide regulatory role in the processes of pregnancy and labour. Furthermore, additional research has suggested the CCAAT/enhancer-binding protein (CEBP) may be another interaction partner for both NF-κB and AP-1 factors. For instance, the gene encoding *CXCL8* (C-X-C motif chemokine ligand 8, also known as IL-8), a prototypic neutrophil chemotactic factor, contains binding sites for AP-1, NF-κB, and CEBP in its promoter, which implies its activation mechanism involves input from these factors (Khanjani et al., 2012). Similarly, the labour-associated *OXTR* promoter region has binding sites for these three factors (Terzidou et al., 2005). These studies suggest that contractility and inflammatory state-driving genes like *OXTR* and *CXCL8* rely upon regulation mechanisms that necessitate the cooperative binding and activation capacities of NF-κB and their interaction partners.

Research into the molecular activities of all four transcription factor families reviewed so far presents a cellular landscape in the myometrium wherein the regulatory pathways contain multiple points of communication between and among their members.

Additional transcription factors

Among the nuclear players implicated in the molecular-level stimulation of myometrial contractility, the aforementioned four transcription factor families have been the most extensively studied to date. However, as well-established transcriptional regulatory networks in other cell types would indicate, the gene regulation mechanisms at work in myometrial tissues throughout the gestational period likely involve additional transcription factors. Indeed, a growing number of recent studies have identified novel factors with marked regulatory effects on labour-associated genes in myometrial cells during their quiescent and/or contractile stages.

Of the lesser-known transcription factors affiliated with the labouring myometrial state, the Krüppel-like factor (KLF) family is perhaps the most noteworthy. KLF9, for instance, is the only novel transcription factor to date whose absence has been shown to result in a prolonged gestation phenotype in homozygous knockout mice (Zeng et al., 2008). At a molecular level, KLF9 has been implicated in the regulation of labour-associated genes *Oxtr* and *Crebbp*, but only on select days broaching the end of the pregnancy period (Zeng et al., 2008). The study reporting these findings rightly acknowledges (within its scope) that the regulatory effects of KLF9 may be directly or indirectly exerted on these target genes; however, more recent work suggests a potential role for KLF factors that would involve their direct engagement with regions of open chromatin in human contractile cells. Wu et al. (2020) observed that regions of open chromatin in the myometrium near term (albeit in non-contractile human tissues) contain a co-enrichment of both KLF- and PR-binding motifs, which lends a possibility of PR and KLF family proteins co-binding to open chromatin during labour. Another study noted a correlation between KLF9 absence and a reduced PRA: PRB expression level ratio at a late gestational stage in a mouse model (Pabona et al., 2015). These findings present KLF9 as a putative strong contributor to the labouring transcriptional regulatory network in both mouse and human myometrium.

Aside from their association with PR isoform activity, KLF factors have been implicated as players in NF-κB-mediated gene regulatory pathways. Both KLF9 and its sister transcription factor, KLF5, have been shown to modify the activity of NF-κB motif-enriched genomic fragments across several *in vitro* assays. For example, Zeng et al. (2008) demonstrated a reduced binding capacity for RELA at NF-κB motifs in the absence of KLF9. Another study observed that, in human myometrial cells treated with IL-1β to simulate a bacterial infection-induced labouring condition, siRNA-mediated reduction of *KLF5* mRNA levels resulted in decreased luciferase activity from an NF-κB motif-laden promoter-carrying reporter construct (Lappas, 2015). More broadly, lowering levels of KLF5 protein in the same system furthermore resulted in reduced expression of key labour-associated cytokines, chemokines and prostaglandins (Lappas, 2015). These observations collectively present a case for at least two members of the KLF transcription factor family acting in tandem with already well-established labour-associated transcription factors to mediate critical transcriptional events during labour.

In addition to KLF9 and KLF5, members of two other transcription factor families have been put forward as pro-labour mediators in an inflammatory context: first, interferon-regulatory (IRF) factors IRF1 and IRF5, and second, Forkhead box protein O (FOXO) factors FOXO1 and FOXO3. Like the aforementioned KLF proteins, these four transcription factors are all more prominently expressed at the mRNA and protein levels in human labouring myometrial tissues compared to non-labouring tissue counterparts (Lappas, 2013, 2015; Lim et al., 2013, 2016, 2018). Lim et al. (2018) have moreover shown that IRF5 can regulate *RELA* promoter-mediated reporter activity *in vitro*, with reduced IRF5 protein levels resulting in decreased luciferase reporter expression in the alternate presence of one of many bacterial protein treatments. Within infection-simulating environments, significant individual depletions of each of the IRF or FOXO factors have been demonstrated to cause a concomitant decrease in levels of select labour-associated cytokines, chemokines and prostaglandins (Lim et al., 2018). Furthermore, IRF factors have been implicated in the increased production of cell adhesion molecules (molecules that, it is worth noting, contain an enrichment of NF-κB motifs in the promoters of their genes) (Lim et al., 2016, 2018). FOXO factors, on the other hand, were shown to mediate expression of critical basal membrane proteins at labour onset (Lappas, 2013; Lim et al., 2013). These experimental observations indicate that IRF and FOXO transcription factor family members may positively regulate a wide array of labour-driving genes in PTL cases associated with intrauterine infection.

Though the aforementioned transcription factors have been tied to contractility-inducing molecular events, some factors belonging to the same family can contribute to the sustention of opposite SMC states. Several studies have implicated the Sma and Mad (SMAD) transcription factor family, for instance, in gene regulatory events in the pregnant or labouring myometrium. Genome-wide analyses of regions that both reside in open chromatin and are bound by PR have uncovered an enrichment of binding sites for SMAD2 in both non-pregnant and term myometrium (Wu et al., 2020); the potential functional contribution of this factor in either myometrial state, however, is yet to be determined. Lim et al. (2017) have shown that SMAD7 is a labour-upregulated protein; furthermore, its targeted reduction in myometrial cells in a bacterial infection-simulating background is accompanied by a concomitant decrease in chemokine, cell adhesion molecule and matrix metalloproteinase production. On the other hand, SMAD3 displays the opposite temporal expression pattern and regulatory effect

on labour-associated genes: reduced SMAD3 levels correlate with increased prostaglandin, cell adhesion molecule and pro-inflammatory mediator synthesis (Lappas, 2018). In this case, despite their structural similarities, the SMAD3 and SMAD7 factors may participate in transcriptional events that underpin the quiescent and contractile myometrial state, respectively.

Members of some transcription factor families also display more complex temporal-specific expression patterns and make different contributions to the SMC phenotype depending on either the gestational stage or the regional location of myometrial tissue. Specificity proteins (SP), factors often grouped alongside the KLF family because of the structural similarities in their domains, fall into this category. An early study implicated select SP factors, including SP1 and SP3, in the regulation of the pregnant state-associated chorionic gonadotropin/luteinising hormone (*CG/LH*) gene, suggesting a combinatorial role for these factors in quiescence promotion (Phillips *et al.*, 2005). However, others have proposed at least partially segregated roles for SP1 and SP3 in the process of labour. While Geimonen et al. (1998) recorded a positive correlation between GJA1 and SP1 protein expression, Pierce and England (2010) conversely suggested that SP1 levels steadily decrease over the course of gestation. This study observed that SP1, when sufficiently up-regulated, can drive the transcription of pregnancy maintenance gene Potassium calcium-activated channel subfamily N member 3 (*Kcnn3*), whose decreased expression at term depends upon accumulated levels of oestrogen (Pierce and England, 2010). Interestingly, whereas SP1 was shown to up-regulate *Kcnn3* promoter-mediated luciferase reporter expression, its ability to do so significantly decreased in the presence of SP3, a sister factor expressed at constant levels throughout gestation. This finding suggests that SP1 and SP3 may compete to determine the expression status of *Kcnn3* (Pierce and England, 2010), and perhaps, other genes. SP factors may therefore mimic the molecular trend displayed by the more well-established transcription factor families: a critical factor expression ratio appears to underpin the formation of putative gestational stage-specific regulatory complexes.

Other transcription factor families are able to exert different functions in the same gestational stage, but across separate myometrial regions within the uterus. Recent studies have identified proteins from the Homeobox A (HOXA) family, namely HOXA10, HOXA11 and HOXA13, as important regulators of uterine smooth muscle function. All three factors may contribute to the establishment of myometrial regionalisation in the pregnant uterus and are thought to control gene expression in SMCs that reside in either the LUS or the FUN. HOXA13, for instance, is expressed at higher levels in the LUS than in the FUN and has the capacity to enhance contractility of human myometrial cells (Liu *et al.*, 2015). At a gene activation level, HOXA13 has been shown to drive increased expression of select inflammatory factors and contraction-associated proteins (including PTGS2 and GJA1) in primary cells isolated from human LUS biopsies (Li *et al.*, 2016). Moreover, in both term and preterm LUS-derived myometrial tissues, HOXA13 can directly up-regulate the expression of prostacyclin synthase (*PTGIS*) and periostin (*POSTN*) genes, likely via the demonstrated recruitment of the factor to the promoters of both genes (Liu *et al.*, 2015). Where HOXA13 is known to exhibit gene-activating potential, the nature of the regulatory roles of HOXA10 and HOXA11 is not as well-established, given the contradictory findings of different studies regarding the functions of these factors. Both HOXA10 and HOXA11 are more highly expressed in the FUN than in the LUS (Liu *et al.*, 2015; Li *et al.*, 2016, 2018). But whereas earlier works correlated HOXA10 levels inversely with progesterone amounts (Cermik *et al.*, 2001) and positively with increased PRA: PRB expression ratios and *ESR1* mRNA levels (Mesiano *et al.*, 2002), a later study presented a different transcription regulatory picture: both HOXA10 and HOXA11 can suppress the expression of select CAP-encoding genes, including *IL-1B*, *IL-6*, *PTGS2* and *GJA1*, in human myometrial cells (Li *et al.*, 2018). Given the discrepancies among these findings, further research into the function of HOXA transcription factors in SMCs from each of the two uterine segments, and, more generally, the regulatory mechanisms responsible for myometrial regionalisation, remain to be conducted.

The validation of gestational stage-dependent or -spanning transcription factor activities in the myometrium collectively illustrates a clearer (albeit still partial) picture of additional players comprising the transcriptional regulatory networks at work during the quiescent and/or contractile stages of the uterine smooth muscle. The majority of the aforementioned studies have focussed on differentially expressed transcription factors in non-labouring and labouring human samples. Interestingly, the murine factor-encoding gene counterparts do not exhibit statistically different expression levels between the two gestational stages; however, the vast majority are highly expressed at both timepoints, with Klf9, Sp1 and Sp3 transcripts situated in the top tenth expression percentile of both pregnant and labouring transcriptomes (Shchuka *et al.*, 2020). In light of their potential shared functional significance across species, it is worth considering how these factors might assemble into broad regulatory units that coordinately promote quiescent and contractile SMC states. Using existing protein-protein interaction data generated from non-myometrial tissues in humans, we attempted to characterise these units. We provided an input list of transcription factors shown to impart some functional contribution to either or both stages into the STRING and GeneMania protein interaction databases (Warde-Farley *et al.*, 2010; Szklarczyk *et al.*, 2019). From the produced output network, we took into account only experimentally validated physical protein-protein interaction data. Based on these results, we introduce a representation of the putative transcription factor protein interaction networks at the pre-labouring and labouring timepoints (Fig. 2). At both stages, interactions are apparent between all input factors, with few additional factors acting as intermediaries between two input proteins. As expected, some factors appeared at both stages, whereas others are restricted to the labouring stage. Additionally, the identification of new transcription factors in these generated interactomes reveals the possibility that other members of the transcription factor families to which the input factors belong may be involved in the same complex as their relatives (see, for instance, IRF8 at the labouring stage). This schematic offers new insight into an expanded repertoire of regulatory proteins that may participate in the transcriptional regulatory networks of the myometrium at particular gestational stages, highlighting additional factors that may prove strong candidates for further labour-associated functional validation.

Conclusion

In this review, we have presented a model of gestation that, the literature shows, is predicated upon the tightly controlled regulation of genes

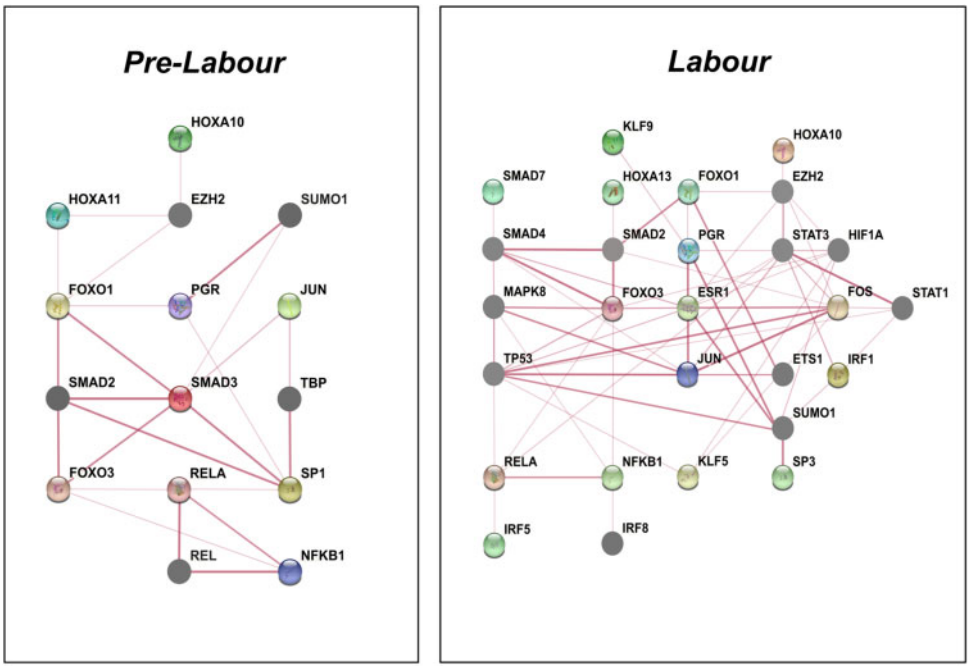

Figure 2. Proposed transcription factor protein interaction network hubs in pre-labouring and labouring myometria. Transcription factors implicated in the regulatory control of gene expression in SMCs were used as input for STRING and GeneMania, two protein-protein interaction databases. Nodes represent transcription factor-encoding genes and associated proteins, with nodes denoting known 3D structures obtained from the STRING database. Input transcription factors are indicated by coloured nodes, and interacting factors are indicated with a grey circle. Red lines represent experimentally validated physical interactions between proteins from at least one species. Increasing line thickness represents higher evidence-based assigned interaction scores, which reflect a measure of the level of confidence in formation of these interactions.

in myometrial cells by a complex network of transcription factors. Critical labour-promoting intranuclear processes, such as the regulation of CAP gene expression, involve contributions from both well-established and more recently identified factors (as modelled in Fig. 3). We have generated a schematic that features a labour-specific transcriptional regulatory network that includes both well-studied transcription factors (AP-1, PRs, ERs and NF-κB), and more recently identified transcription factors (KLF9, KLF5, IRF1, IRF5, FOXO1, FOXO3, SMAD7, HOXA10, HOXA13, SP3). The specific types of interactions and interaction partners adjoining these factors likely guide the switch of the myometrium from the quiescent to the contractile state. These factors may bind at the promoters and, in some cases, at distal regulatory regions that control the transcription of labour-associated genes. These regulatory regions are accessible and display active histone marks, two features that would facilitate the binding of transcription factors. Subsequent recruitment of RNAPII to promoters of critical genes allows for initiation of transcription throughout gene bodies. The transcription regulatory networks we have proposed above offer a preliminary glimpse into the identity of molecular players within complexes that may bind regulatory regions in gestational stage-defining genes, allowing for the stage-specific regulation of gene expression in the myometrium.

Identifying the full repertoire of these players becomes increasingly significant given that transcription factors have become attractive targets for novel therapeutic approaches in multiple disease contexts. Existing treatments for PTL, which act through a mechanism that hones in on sub-cellular components apart from transcription factors, are insufficiently effective. These immunomodulatory agents overwhelmingly consist of non-steroidal anti-inflammatory drugs (NSAIDs) or other tocolytic agents meant to regulate inflammation- and immune response-affecting genes such as those encoding cytokine receptors (De Bosscher *et al.*, 2003). Some examples include treatment with: indomethacin, which poses an inhibitory effect by reducing the expression of the pro-labour gene, prostaglandin; nifedipine, which blocks the labour-associated calcium ion influx into myometrial cells (King *et al.*, 2003); and magnesium sulphate, which competes with calcium for binding at motor end plates and/or voltage-gated channels. However, these treatments can have adverse effects on maternal and foetal health (Haas *et al.*, 2012). Additionally, none of these treatments are capable of delaying labour for longer than at most 7 days, which is not enough time to prevent the risk of neonatal complications in many preterm birth cases (Abou-Ghannam *et al.*, 2012).

Targeting proteins involved in gene expression regulation may instead pose a more promising alternative solution. Although some transcription factors lack classic-binding pockets for synthetic molecules that can bind and alter factor activity, recent studies in cancerous disease contexts have revealed that at least some may qualify as therapeutic molecular targets (as reviewed in Lambert *et al.*, 2018). Transcription factor-targeting drugs may work at multiple levels within

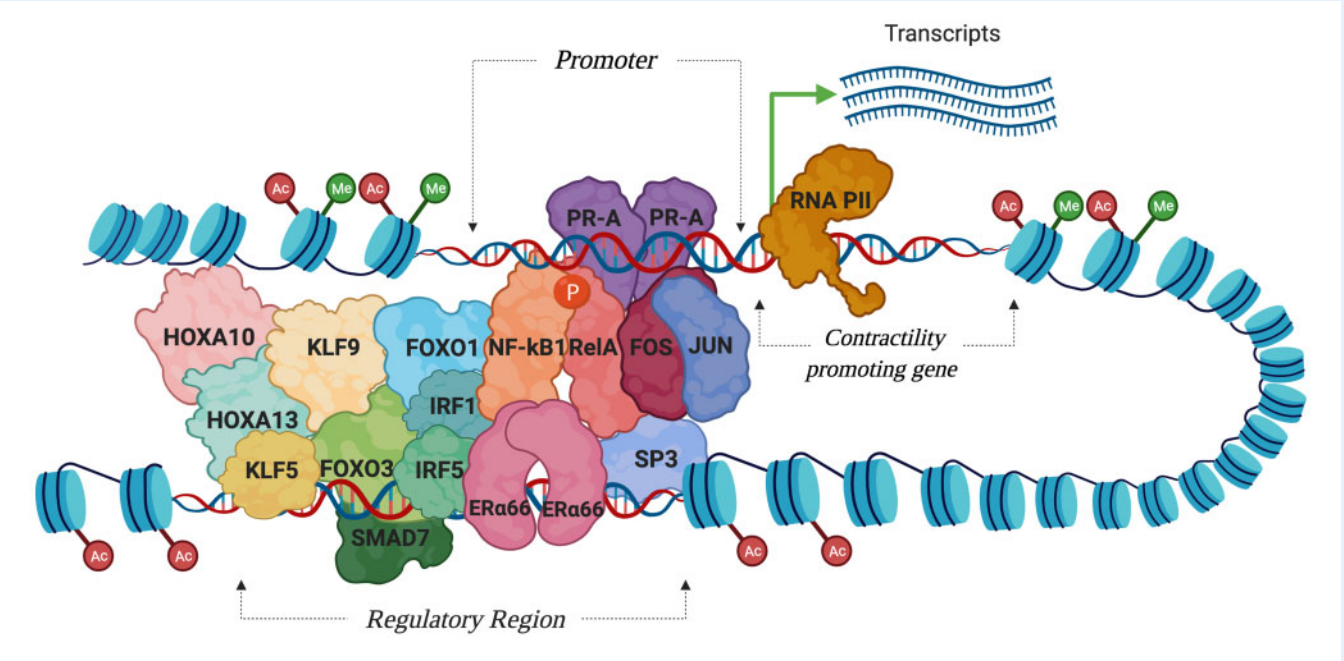

Figure 3. Model of contractility-promoting gene up-regulation by key players belonging to the putative labour-specific transcriptional regulatory network in the myometrium. We propose that labour-associated gene expression control depends upon recruitment of a multi-protein complex containing transcription factors (TFs) and RNA Polymerase II (RNAPII) to the promoter. This recruitment allows for ensuing active transcription of genes necessary for labour onset. TFs displayed in this model, i.e. those deemed critical in labour-associated gene induction, include both well-characterised factors (AP-1 heterodimers (FOS: JUN), PR homodimers (PR-A), ER heterodimers ERa66 (the full-length receptor) and ERΔ7 (the shorter isoform), and NF-κB heterodimers (RELA-NFKB1)) as well as more recently studied TFs (KLF9, KLF5, IRF1, IRF5, FOXO1, FOXO3, SMAD7, SP3, HOXA10, HOXA13). We suggest that these transcription regulatory complexes bind at promoters and, in certain gene contexts, at distal regulatory regions of contractility-promoting genes. Both types of regions are depicted here, including markers of open chromatin features, such as histone methylation (Me, H3K4me3) and acetylation (Ac, H3K27ac), that allow for active transcription. While this model depicts a full repertoire of regulatory TFs binding at critical genomic regions, not every contractility-promoting gene may be mediated by all factors; some genes may instead be regulated by only a subset of these TFs. Created with BioRender.com.

the expression and action pathways of these proteins through a variety of mechanisms. For instance, transcription factor-encoding gene expression in the nucleus may be activated or inhibited. Alternatively, transcription factor degradation pathways can be targeted, as in the case of the mebendazole drug (a.k.a Vermox®), which drives the elimination of the MYB transcription factor to treat acute myeloid leukaemia (see Matthews *et al.*, 2015). Furthermore, the ligand-binding ability of select transcription factors can be exploited; for instance, tamoxifen is administered to compete with oestrogen to bind ERs in breast cancer cells and its application has been shown to reduce breast cancer reoccurrence as well as contralateral breast cancer rates by 40–50% (see Early Breast Cancer Trialists' Collaborative Group (EBCTCG) *et al.*, 2011). Finally, transcription factor protein-protein interactions or factor binding to DNA can be blocked. The mithramycin drug family, for example, prevents the binding of SP1/3 transcription factors to regulatory regions across several oncogenes in HeLa and nasopharyngeal carcinoma cell lines (Ray *et al.*, 1989; Zhang *et al.*, 2014). These findings all hold an exciting potential with regard to the development of powerful measures to prevent preterm birth.

Even if more effective preventative methods can be developed with a distilled list of targetable transcription factors, however, we cannot expect that such methods could be applied successfully in every PTL case. Several challenges face the generation of methods that could assist all women at risk for preterm birth. The multi-factorial causal nature of PTL suggests women can deliver infants prior to term for a variety of reasons. Furthermore, preliminary results with animal models have not always had direct applicability to human cases (reviewed in Mitchell and Taggart, 2009). Therefore, despite significant research advances, further study into the molecular basis of the process of parturition, specifically with regard to the genome-level changes that guide the myometrium towards a contractile state, is required. If tentative solutions, such as the implementation of proteomics and genomics techniques to create gene profiles of PTL subclasses to provide tailored therapies (Esplin, 2006), are to be developed to address these challenges, one way forward remains clear: arriving at a clearer picture of the critical molecular processes involved in the induction of labour.

Data availability

No new data were generated or analysed in support of this research.

Authors' roles

The literature search was conducted by NK, VMS and OS. All authors contributed to the writing of the manuscript. NK and JAM created the final figures. All authors revised the manuscript and approved the final version.

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Conflict of interest

The authors declare no conflict of interest.

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