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Abstract

Microarray technologies widen our comprehension of the major structural and metabolic transformations which affect the myometrium from the very beginning of pregnancy until parturition. The results are coherent with the mass of information which was accumulated previously, primarily on the basis of studies of selected critical factors. They highlight the activation of precise signaling pathways, some of which may have been previously under evaluated. The remodelling and maturation processes that the myometrium undergoes in pregnancy appear clearly as phenomenon's which last during the full course of gestation. Comparatively, the onset of labor is perhaps the phenomenon which remains the least well described by these methods of analysis. Nevertheless, genomic studies constitute a necessary first step of orientation and help establishing new links between the generic signaling pathways that are activated during the normal or pathological gestation. These studies also represent an indicative step that will have to be paralleled, in the future, with the results of the systematic proteomic analysis of the myometrium.

Introduction

Parturition encompasses composite physiological processes that require synchronization of uterine contractions, cervical dilatation and fetal membrane rupture. The mechanisms by which parturition is triggered remain unknown but they are, as all developmental processes, the results of timely coordinated biochemical and physiological steps. Over the past decades, reductionist approaches have investigated the cellular and molecular bases of these developmental changes and focused on assessing the regulation of selected critical factors involved in the onset of labor.

Recently, the near-entire genomic sequence of the human and several model animals have provided the opportunity to enhance our understanding of the relationships between genes, phenotypes and global transcriptional status. Oligonucleotides or DNA microarray technologies allow the examination of the function of thousands of genes at once and in parallel, thereby providing an “assay” of the transcriptional status of cells or tissues in a wide variety of physiological or pathophysiological situations (Dunckley *et al.*, 2005). In the context of uncomplicated or complicated pregnancy and parturition, their interest is to obtain a molecular snapshot of the expression profile of gene transcripts as a function of the time-dependent process regulating myometrial activity.

Functional genomic studies during pregnancy

Functional genomics has arrived at the study of parturition in different species: mice (Bethin *et al.*, 2003, Salomonis *et al.*, 2005), rats (Girotti & Zingg, 2003), human (Bethin *et al.*, 2003, Chan *et al.*, 2002, Charpigny *et al.*, 2003, Esplin *et al.*, 2005, Havelock *et al.*, 2005, Keelan *et*

al., 2003, Marvin *et al.*, 2002a, 2002b, Tashima *et al.*, 2002), and different tissues: myometrium (Aguan *et al.*, 2000, Bethin *et al.*, 2003, Chan *et al.*, 2002, Charpigny *et al.*, 2003, Esplin *et al.*, 2005, Havelock *et al.*, 2005, Salomonis *et al.*, 2005), fetal membranes (Chen *et al.*, 2002, Keelan *et al.*, 2003, Marvin *et al.*, 2002a, 2002b, Muhle *et al.*, 2001, Tashima *et al.*, 2002), trophoblast (Chen *et al.*, 2002, Kato *et al.*, 2002). Various methodologies have been used: Suppression Subtractive Hybridization (SSH) (Chan *et al.*, 2002) or microarrays for most of the previous cited studies. All investigators have reported genes differentially regulated during parturition (Romero *et al.*, 2002) and multiple novel candidate markers for preterm labor (Chan *et al.*, 2002, Marvin *et al.*, 2002a, 2002b, Wu *et al.*, 1999) or for premature rupture of membranes (Tashima *et al.*, 2002). They contributed to shed light on specific pathophysiological issues like the patterns of expression of cytokines in the fetal membranes and decidua particularly in the presence of intrauterine infection (Keelan *et al.*, 2003) or on the induction of enzymes for prostaglandin synthesis (Bethin *et al.*, 2003). Groups of genes with yet unknown functional connection to these pathologies were also found coordinately expressed. This was emphasized in animal studies which allow to explore the global pattern of gene expression and their co-regulation on the basis of their genomic location over the full time-course of myometrial transformation (i.e. pregnancy) or following experimentally-controlled infection (Girotti & Zingg, 2003, Muhle *et al.*, 2001, Salomonis *et al.*, 2005, Wu *et al.*, 1999).

Computational methods have significantly helped to the interpretation of gene profiling experiments by delineating clusters of genes sharing coherent expression features (Claverie, 1999). During the last five years, statistical methods and data analysis for array studies have progressed enormously and today an “ideal” study should now incorporate i) careful research designs (differential expression or cross sectional studies), ii) statistical methods

incorporating background adjustment and normalization of data, assessment of differential expression after determining sample size to control the proportion of positive calls that are false positives (False Discovery rate), iii) hierarchical clustering (e.g. HOPACH method) (Ganesh *et al.*, 2004, Salomonis *et al.*, 2005), iv) functional organization of genes into pathways networks with the aid of integrated databases like the Gene Ontology consortium (Lewis, 2005) or the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa, 1997, Kanehisa & Goto, 2000) and finally v) integrating genotype, transcription and clinical trait data, e.g. see (Salomonis *et al.*, 2005).

The biases adopted in this review are several. We focused attention on genes expressed essentially in the “normal” human myometrium. One study has reported a direct comparison of the non-pregnant myometrium (NP) with the pregnant human myometrium at term not in labor (TNIL) (Rehman *et al.*, 2003). It gives an overall picture of the changes that the uterine muscle undergoes in its adaptation to the gestation. Two studies have described the changes in gene expression in the myometrium in preterm patients not in labor (PT) versus patients at term not in labor (TNIL) (Bethin *et al.*, 2003, Charpigny *et al.*, 2003) and two additional studies have described the changes in gene expression in the myometrium in patients at term not in labor (TNIL) versus patients at term in labor (TIL) (Esplin *et al.*, 2005, Havelock *et al.*, 2005). Other studies have not been compiled directly, either because they did not provide sufficient precise references on the genes being studied (i.e. lack of GenBank or Unigene references), they considered other components of the utero-fetal unit (i.e. membranes, decidua, cervix, etc.) or they were carried out on animal species. To translate lists of tens or hundreds of genes found to be differentially regulated in the conditions under study into a clearer understanding of the biological phenomena involved, we used both combinations of searches through the literature referenced in public databases and the Onto-Tools software

developed by the Draghici's group at Wayne State University, Detroit (MI), (http://vortex.cs.wayne.edu/Projects.html). The Onto-Express module helps recognizing functional profiles (using Gene Ontology terms) for the categories: biochemical function, biological process, cellular role, cellular component, molecular function, and chromosome location (Draghici *et al.*, 2003). The Pathway-Express module helps data mining, by proposing on the basis of a computational method a hierarchical list of several KEGG pathways (Kanehisa, 1997, Kanehisa & Goto, 2000) most likely adjusted to changes observed in microarray experiments (Khatri *et al.*, 2005). Drawbacks and limitations inherent to the use of these tools or of their cognate alternatives for ontological analysis, have been reviewed recently (Khatri & Draghici, 2005).

The variations of myometrium gene expression in the course of gestation could be dispatched in 14 main KEGG pathways (http://www.genome.jp/kegg/pathway.html) that were the most representatives for the changes seen in non-pregnant (NP) versus term quiescent pregnant uterus (TNIL), quiescent preterm (PT) versus term quiescent uterus (TNIL) and term quiescent uterus (TNIL) versus contracting uterus at labor (TIL). Tables 1-4 and Figures 1-2 summarize the compiled data which are commented below in more details. Wherever possible, OMIM nomenclature has been adopted in the text and tables (http://www.ncbi.nlm.nih.gov/omim/).

Changes in structural and contractile genes during gestation

Actin cytoskeleton, focal adhesion, adherens and tight junctions related genes represent a large subset of genes that are over-expressed in the pregnant human myometrium as compared to the non-pregnant state (Table 1).

Regulation of actin cytoskeleton

Growth and cytoskeletal remodeling of myometrial cells during pregnancy are critical for myometrial functions including those expressed during labor and delivery. At term, signals that initiate labor, ultimately promote a switch in the phenotype in the quiescent uterus to a smooth muscle which becomes spontaneously active, excitable, highly responsive to uterine agonists, and exhibits a high degree of cell-cell coupling. Myometrial cells are rich in actin microfilaments, intermediate filaments and microtubules that allow cells to adapt to a variety of shapes and to carry out coordinated and directed movements. In rat, myometrial expression of alpha-actin is high throughout pregnancy. An increased expression of gamma-actin and its translocation to the membrane is observed in uterine myocytes at late gestation. Therefore, the alteration in myometrial composition of contractile proteins is important to prepare the myometrium for the development of contractions during labor (Shynlova *et al.*, 2005).

RhoA, a member of the Ras superfamily, and its downstream mediator RhoA kinase (ROCK1) are necessary for agonist-induced stress fiber formation (Gogarten *et al.*, 2001). The higher expression of protein kinase C (PKC) isoforms observed in pregnant *versus* non-pregnant myometrium (Rehman *et al.*, 2003) may promote or drive the formation of stress fibers. Additionally, up-regulation of ROCK1 has been demonstrated in human term myometrium (Moore *et al.*, 2000, Rehman *et al.*, 2003). This leads to the idea that increased endogenous ROCK1 activity, resulting in enhanced RhoA-mediated calcium sensitization, is involved in the increased contractility at the time of labor. It is of note that EGF increases the presence of actin and myometrial EGF receptors transcripts are increased at the time of parturition (Charpigny *et al.*, 2003, Gargiulo *et al.*, 1997, Rehman *et al.*, 2003).

Focal adhesion

The transmission of force between the contractile apparatus of the cell and extracellular matrix (ECM) occurs at membrane-associated dense plaques or “focal adhesions”. Focal adhesions consist of clusters of integrins that mediate interactions between the extra- and intracellular environments. The cytoplasmic regions of integrins connect with cytoskeletal elements and signaling components such as Focal Adhesion Kinase (FAK), while the extracellular regions connect to specific extracellular matrix molecules such as collagen, laminin or fibronectin.

Fetal growth imposes mechanical tension on the myometrium at term, which in turn induces activation of FAK leading to focal adhesion turnover and supporting myometrial cell hypertrophy. During late pregnancy, a fall in tyrosine phosphorylation of FAK and stabilization of focal adhesions occur for provoking the cessation of myometrial hypertrophy. Because actin polymerization and the dynamic remodeling of the actin cytoskeleton play key roles in the regulation of myometrial contraction, there is growing evidence that stretching induce labor, probably through a change in the expression of contraction-associated-proteins (CAPs) (Macphee & Lye, 2000). In human term pregnant myometrium, changes in cytoskeletal organization support a role for FAK and other focal adhesion-associated proteins as regulators of actin dynamics. ITGA5 with its known partner ITGB1, is up-regulated in rat myometrium during late pregnancy and labor (Williams *et al.*, 2005) as well as ITGAV in the TNIL human myometrium (Rehman *et al.*, 2003). Therefore they may interact with actin-binding proteins (e.g. actinin and filamin) to form mechanical links to the cytoskeleton. A strong expression of vinculin is concentrated at actin-vinculin focal adhesion sites in human

myometrial cells (Yu & Lopez Bernal, 1998). At the time of parturition, expression of integrins declines and the focal adhesion and related pathways do not change in studies which compare the TNIL and TIL states of pregnancy (see Table 1).

Parturition as an inflammatory process

Cytokine-chemokines interaction

There is a massive influx in the myometrium of neutrophils, macrophages and T-lymphocytes concomitant with the onset of labor (Osman *et al.*, 2003). An increase in cytokines, interleukin-1 (IL-1), IL-6, IL-8 and tumor necrosis factor (TNF)-alpha within tissues of the laboring uterus and cervix is demonstrated.

Chemokines enhance inflammation by inducing chemotaxis and cell activation of inflammatory cells. CXC-Chemokines, attract neutrophils but not macrophages, while CC-Chemokines preferentially induce migration of macrophages. Chemokine transcripts increase in myometrium at term, including CCL13 (MCP-4 or Monocyte chemotactic protein-4), CCL19, CCL21, CXCR4 (NPYRL or neuropeptide Y receptor-like) and CXCR5 (Burkitt lymphoma receptor) (Bethin *et al.*, 2003, Charpigny *et al.*, 2003, Rehman *et al.*, 2003). At the time of labor, a selective increase in CXCL10 (Interferon-inducible protein-10), CCL8 and CC13 is noted (Esplin *et al.*, 2005) (see Table 2). IL-8, MCP-1 and RANTES are regulated by local growth factors and cytokines such as TNF-alpha, interferon-gamma, and IL-1. IL-8 also potentiates the effect of IL-1-induced myometrial contractions through PGE2 production at the time of parturition and up-regulates myometrial TGF-beta receptors expression in vitro, suggesting an additional autocrine-signaling pathway. Therefore, it is clear that coordination

of chemokine-chemokine receptor interactions plays an important role in successful pregnancy (Kayisli *et al.*, 2002).

The TNF receptor super family member Fas and its cognate ligand (FasL) play a role in cell leiomyoma apoptosis (Wang Y. *et al.*, 2002). Therefore increased expression of TNFRS6 (i.e. Fas receptor) in term myometrium (Table 2) await consideration in the control of the uterine growth process. Elevated expression of TNFRS11B is observed in human myometrium with or without labor (Esplin *et al.*, 2005, Rehman *et al.*, 2003). A peak in uterine osteoprotegerin (i.e OPG or TNFRS11B), a soluble membrane of the TNF-alpha receptor family that acts as a negative regulator for receptor activator of nuclear factor-kB (RANK), has been reported during labor in the rat (Girotti & Zingg, 2003).

The gp130 protein is a subunit component of several cytokines receptors including those for LIF. Cytokines sharing the gp130 (or IL6ST) subunits are referred to as IL6 type family of cytokines and signal through JAK/STAT pathway (Heinrich *et al.*, 1998). They play a role in the regulation of gene activation, proliferation and differentiation. Down-regulation of IL6ST and LIF receptor at term (Table 3) may be an indication of the decrease of proliferation processes before the onset of labor.

IFN-gamma inhibits the proliferation of vascular smooth muscle cells and the synthesis of collagens by myofibroblasts. In human myometrial cells, interferon gamma antagonizes IL-1beta-induced prostaglandin-endoperoxide synthase 2 (COX-2 or PTGS2) expression and PGE2 production (Hertelendy *et al.*, 2002). Therefore, the decrease in IFNGR1 transcript observed during the transition from PT to TNIL states of the uterus (Table 2), could be interpreted as an *ad hoc* evolution for labor onset.

TLR signaling pathway

Toll-like receptors (TLRs) are evolutionarily conserved pathogen-associated microbial patterns and play important roles in innate immunity in mammals. Ten TLRs are evidenced in the uterus but TLR2 and TLR4 mRNA are expressed at the highest levels. Among the TLR-related gene transcripts, SOCS, CD14 and IRAK mRNAs (see Tables 2 and 4) are widely expressed in the myometrium (Nishimura & Naito, 2005). TLR4 mediates induction of PTL in mice treated with lipopolysaccharide (LPS) (Wang H. & Hirsch, 2003). In monocytes, LPS-induced signaling through TLRs, lead to the recruitment of docking proteins such as IL-1 receptor-associated kinase (IRAK) and TNF-alpha receptor-associated factor (TRAF6) leading to the activation of IkappaB kinase (IKK) complex. These pathways in turn activate transcription factor such as NF-kB that coordinates the induction of genes encoding inflammatory mediators (Guha & Mackman, 2001). In human pregnant myometrial cells a positive immunoreactivity for TLR4 is observed and cells exposure to LPS lead to nuclear translocation of the p65 subunit of NFkB (Dallot *et al.*, 2005). The increased expression of CD14 together with increased expression of the catalytic subunit of PI3K in pregnant myometrium may serve to increase host protection against microbial invasion (Rehman *et al.*, 2003). At the end of pregnancy, because some of the TLR-related genes such as IRAK, IKK, MKK have decreased expression compared to earlier stage of the gestation (Charpigny *et al.*, 2003), it is possible that adaptative responses to immune or growth processes take place.

TGF-beta signaling pathway

TGF-beta regulates its own expression, the expression of ECM, of matrix metalloproteinases (MMP), and of tissue inhibitor of matrix metalloproteinases (TIMP) as well as the growth of leiomyomas and of normal myometrial cells (Chegini *et al.*, 1999). TGF-beta receptors mediate their action through multiple pathways, including smad proteins that convey TGF-beta receptor signals from the cell surface to the nucleus, resulting in transcriptional activation of TGF-beta-responsive genes. TGF-beta also activates mitogen-activated protein kinase (MAPK), PKC, and calcium/calmodulin complex, inducing Smad-independent transcriptional responses. One mechanism by which TGF-beta is activated involves thrombospondin (THBS1). THBS1 promotes replication of smooth cells and modifies responses to contractile agents. THBS1 expression and protein in human myometrial tissues are increased during labor and after the administration of OT (Morimoto *et al.*, 1998). Myometrial transcripts of THBS1 and THBS2 increases during pregnancy (Charpigny *et al.*, 2003, Rehman *et al.*, 2003) and peak at the time of labor concomitantly with the expression of TGF-beta (Esplin *et al.*, 2005). It is possible that the control of the TGF-beta pathway in the near term myometrium facilitates the transition of the quiescent uterus towards a contractile state, as well as it contributes to control the uterine growth.

Wnt signalling pathway

Leiomyomas have high levels of both transcripts of WNT5B and of secreted frizzled related protein 1 (SFRP1), a modulator of Wnt signaling (Mangioni *et al.*, 2005). Strong SFRP1 expression under high estrogenic conditions seems to contribute to the development of uterine leiomyomas through the antiapoptotic effect of SFRP1 (Fukuhara *et al.*, 2002). The increased expression of WNT5B in the pregnant myometrium suggests that the Wnt pathway is important in myometrial adaptation to pregnancy by decreasing apoptotic myometrial cell

death (Rehman *et al.*, 2003). Inversely, other genes associated with the inhibition of cell proliferation such as p53 are increased at the end of gestation (Charpigny *et al.*, 2003).

Kinases located at the crossroad of uterine contractility and myometrial cell proliferation

MAPK-signaling pathway

MAPK signaling cascades regulate diverse processes ranging from contraction, proliferation, differentiation, and development. Five families of MAPKs have been defined in mammalian cells: i) extracellular signal-regulated kinases (ERK1 and ERK2), ii) Jun N-terminal kinases (JNK), iii) p38 kinase isozymes. ERK1 and ERK2 are activated by mitogenic stimuli such as growth factors, cytokines and phorbol esters. Members of the JNK family play crucial roles in regulating responses to various stresses and apoptosis. Among the targets of p38 MAPKs are several transcription factors, including NF-κB, p53 and ATF2, which modulate the expression of genes encoding inflammatory cytokines (Qi & Elion, 2005). MAPKs are involved in inhibiting gap-junction-mediated cellular communication in rat myometrium (Loch-Caruso *et al.*, 2003). Mechanical stretch of the rat uterus stimulates myometrial cell hypertrophy (Douglas *et al.*, 1988) via a mechanism involving integrin/focal adhesion/MAPK cascades (Macphee & Lye, 2000). Activation of MAPKs is necessary for optimal stretch-induced c-fos expression (Oldenhof *et al.*, 2002). In addition, the spatial expression of MAPK p38 and ERK-1/2 in conjunction with ATF2 isoforms within the uterine corpus during pregnancy and labor is likely to be important in preparation of the uterus for labor (Otun *et al.*, 2005).

In the myometrium, the MAPK pathways are also implicated in the induction of PTGS2 expression (Bartlett *et al.*, 1999, Sooranna *et al.*, 2004) and in mediating the effects of prostaglandins (PGs) (Ohmichi *et al.*, 1997), OT (Zhong *et al.*, 2003), endothelin-1 (ET-1) (Kimura *et al.*, 1999) and corticotropin-releasing hormone (CRH) (Grammatopoulos *et al.*, 2000, Papadopoulou *et al.*, 2004). Increasing maternal plasma levels of CRH during the last weeks of pregnancy and the substantial expression of CRH receptors in choriodecidua, placenta and myometrium suggests that this stress hormone plays a role in the control of human parturition (Sehringer *et al.*, 2004).

MAPK activity increases in the rat myometrium from day 15 to day 20 of gestation and declines sharply just before parturition (Robin *et al.*, 2004). During the same interval, a shift in intracellular distribution of the Ras protein precedes the down-regulation of membrane-dependent mitogenic signaling and uterine hypertrophy as pregnancy approaches parturition (Ruzycky, 1998). During RU-486-induced PTL, as previously described for spontaneous labor, ERK phosphorylation levels increase, as does phosphorylation of caldesmon and of 20-kDa MLC. When rats are chronically treated with an agent that prevents ERK activation, the onset of PTL is delayed (Li *et al.*, 2004, Liu *et al.*, 2004).

OT-mediated ERK1/2 activation in myometrial cells involves a phospholipase C (PLC)-independent pathway (Zhong *et al.*, 2003). A cross-talk between growth factors receptors and the estrogen receptor alpha (ESR1) has been proposed. The estrogen response involves estradiol (E2)-ESR1-mediated responses as well as responses resulting from convergence of growth factor and ESR1-initiated activities (Hewitt *et al.*, 2005). Functional signaling proximal to IGF-1R is maintained in the ER alpha-KO mouse uterus. ER alpha is necessary

for IGF-1 induction of uterine nuclear proliferative responses, and a cross-talk between IGF-1R and ER signaling pathways exists *in vivo* (Klotz *et al.*, 2002).

Protein kinase C

PKC constitute a multigene family located at the crossroad of two essential uterine functions, namely contractility and cell proliferation. Six isoforms of PKC, the conventional PKC isoforms (alpha, betaI, betaII, and gamma), the novel PKC isoforms (delta, epsilon, theta, eta, lambda/iota) and the atypical PKC isoform (zeta) are evidenced in the human pregnant myometrium. Whereas PRKCA is required for proliferation of human myometrial cells (Eude *et al.*, 2002), only activation of PRKCZ results in actin reorganization and elicits contractions of the human myometrium at the end of pregnancy (Di Liberto *et al.*, 2003).

A balance between T helper (Th1) (pro-inflammatory) / Th2 (anti-inflammatory) cytokine production, has been described at the time of parturition, and a link between TNF-alpha/IL1-beta and premature human childbirth has been proposed (Arntzen *et al.*, 1998). Because, atypical PKC's are important components of the TNF/IL1B signaling pathway that controls NF-κB activation, the implication of the PRKCZ in the control of the onset of labor in women is not surprising. (Ozaki *et al.*, 2003) also demonstrated that the levels of mRNA of PRKCB1 isoform in pregnant human myometrium were greater than those in nonpregnant myometrium, a feature confirmed in genomic studies (Table 3) (Rehman *et al.*, 2003). The levels of the phosphorylated substrate for PKC, CPI-17 which is considered to inhibit myosin light chain phosphatase were also greater in the pregnant myometrium. These results suggest that the PKC-mediated contractile mechanism is augmented in human

myometrium after gestation, and that this augmentation may be attributable to the increased activity of the PRKCB1 isoform and CPI-17.

Phosphatidyl inositol signaling pathway

The PI3-kinase cascade is activated through the binding of the regulatory subunit to phosphorylated tyrosine residues, leading to enhanced activity of the catalytic subunit. PI3 kinase phosphorylates phosphatidyliositol-4,5-bisphosphate (PIP2) to generate PIP3 which interacts with protein kinase Akt or protein kinase B (PKB). Activation of Akt influences many cellular functions through PIP3 binding and phosphorylation by phosphoinositide-dependent kinase 1(PDK1). It includes cytoskeletal organization, cell growth, motility, proliferation and survival. PDK1 is known to phosphorylate PKC-zeta in the activation loop. PI3 kinase is also activated by direct binding of the catalytic subunit to activated Ras and PI3 kinase cascade activation can lead to the activation of the ERK cascade (Stein & Waterfield, 2000).

Increases in $[Ca^{2+}]_i$ are controlled by multiple signal pathways in myometrium. GPCR-mediated stimulation of the Gq/11 subfamily and subsequent activation of PLC subfamily results in generation of IP3, which triggers release of Ca^{2+} from intracellular stores. Five PLC isoforms: beta 1, beta 2, beta 3, gamma 1, and gamma 2 are detected in human myometrium. OT activates human myometrium by interacting with at least two G proteins and possibly three PLC beta isoforms (Phaneuf *et al.*, 1996). Indeed, the amount of PLCB4 is increased at midpregnancy, whereas PLCB1, PLCB2, and PLCB3 are up-regulated at term in the rat uterus (Mhaouty-Kodja *et al.*, 2004). PLCB3 may be targeted by both contractant and relaxant signaling pathways in myometrium and play a critical role in the balance between

them (Zhong *et al.*, 2005). Both DAG-sensitive PKC, activated by PLCB products, and DAG-insensitive PKC, possibly activated by PI3kinase-dependent process are involved in ERK activation to modulate rat uterine functions (Robin *et al.*, 2002). In addition, protein tyrosine kinase/phosphatase activities may control both phosphorylation and activation of PLCG1 and contribute to the modulation of the generation of inositol phosphates and uterine tension.

Genomic studies exploring the transition from the NP to the TNIL state found an up-regulation of the transcripts pertaining to the phosphoinositol signaling pathway (Rehman *et al.*, 2003), whereas those comparing the PT to the TNIL state found a down-regulation of transcript expression (Bethin *et al.*, 2003, Charpigny *et al.*, 2003). This may be interpreted as a slowdown of myometrial cell proliferation processes when pregnancy comes close to term (Table 3).

Apoptosis and parturition

Proliferation of smooth muscle cells and fibroblasts occurs in early pregnancy and decreases as the uterus nears parturition. In late pregnancy and during involution of the rat uterus, an increase of apoptosis is observed (Leppert, 1998). However, the extent of apoptosis or dedifferentiation that may occur in human myometrial cells during post-partum involution is still unknown. The most investigated factors linked to apoptosis are the Fas ligand (FasL), Fas receptor (FasR), the TNFalpha and its receptors. Some of the genes associated with the inhibition of cell proliferation and differentiation are increased in the myometrium at the end of pregnancy (Table 4). For an example, the TNFR6 or Fas antigen and the p53 tumor suppressor limit cellular proliferation by inducing cell cycle arrest and apoptosis. The presence of DFFA (DNA fragmentation factor-45), known to be essential for chromatin

condensation during normal apoptosis and the inhibition of the PI3kinase in the term pregnant myometrium, may also contribute to an increase of apoptotic events.

G-protein signaling and parturition

The silencing of the myometrial contractile function is conditioned by a predominant functional cAMP/cGMP system, whereas contractions are under the control of agonist-induced calcium mobilization via the PLC pathway. Gestational-related modifications of GPCR in the myometrium, as well as changes in their associated kinases (GRK), cognate G proteins, and effectors have been detected (Hertelendy & Zakar, 2004, Lopez Bernal & TambyRaja, 2000). Some GPCRs coupled to PLC generates the second messengers IP3 and DAG. The ensuing rise in IP3 releases Ca^{2+} from the sarcoplasmic reticulum, causing a sudden rise in intracellular calcium, whereas DAG activates PKC and the MAPK cascades. Uterine contractility can also be enhanced via the activation of Rho GTPases, and the subsequent action of ROCK's to potentiate the effect of myosin light chain kinase (MYLK). These pathways have been discussed above. Other GPCR coupled to the adenylyl cyclase promotes the accumulation of cAMP which controls the relaxation of the myometrium during pregnancy via the inactivation of MYLK and the activation of ATF-2 (Bailey & Europe-Finner, 2005). Another powerful way for increasing cAMP concentration consists in inhibiting its hydrolysis by phosphodiesterases (PDEs). Among the five PDE families (PDE 1, 2, 3, 4 and 5) identified in the human myometrium (Leroy *et al.*, 1994, Leroy *et al.*, 1999), one particular isoform, PDE4B2, which specifically hydrolyses cAMP is selectively induced at the end of pregnancy suggesting a role for this protein in the setting of the contractile state of the myometrium just before delivery (Mehats *et al.*, 2001, Mehats *et al.*, 2000).

Neuroactive-ligands and parturition

A small selection of peptides, neuroactive factors and ion channels such as ET-1, noradrenaline, NPY, PGE2, GABA… that mediate uterine relaxation or contraction and act through GPCR are mentioned here (see Table 4). ET-1 exerts a stimulatory effect on myometrium contractility and proliferation. Its transcript is up-regulated in the pregnant myometrium (Rehman *et al.*, 2003). Among the three subtypes of alpha2-adrenoceptors found in the human myometrium at term pregnancy (Adolfsson *et al.*, 1998), only the alpha2C-subtype appears to be predominant in pregnant myometrium. Stimulation of GABA (A) receptors tonically inhibits contractions of the rabbit myometrium, while stimulation of GABA (B) receptors enhances contractions. Steroids interact with GABA (A) receptors to modulate uterine contractility (Majewska & Vaupel, 1991). The subunit composition of GABA (A) receptor differs in rat uteri throughout gestation and just before labour in humans, a decline in GABA receptor transcripts is observed (Bethin *et al.*, 2003, Charpigny *et al.*, 2003). TRH-R is expressed in term myometrium. (Fukusumi et al., 1995) have previously reported high level of TRH-R mRNA in uterus but whether TRH plays an important role in the female reproductive tract remains to be elucidated. PGs, in conjunction with their numerous receptors activate multiple signaling pathways and exert multifaceted actions in myometrium (Hertelendy & Zakar, 2004). Thus, EP3 transcripts are down-regulated in the term pregnant myometrium (Bethin *et al.*, 2003, Charpigny *et al.*, 2003). However, the significance of these changes remains to be established, because of the known existence of EP3 splice variants.

Future strategies for integrative analysis of myometrial functions

A question is what could be the composition of an “ideal” genomic array for the study of parturition? The answer is not unique. Undedicated or "generic" microarrays seem heuristically more potent to explore and detect new interesting metabolic pathways than specialized arrays could do (Tierney *et al.*, 2003). Inversely, a myometrium-dedicated microarray is unlikely to provide essential clues on the mechanisms of parturition for the following reasons.

Gene module analysis, as exemplified in this review, searches for coordinate regulation of genes that belong to a priori defined gene modules. A statistical test performed for each module relative to all other genes on the microarray calculates whether the degree of coordinate regulation is more than one would expect by chance. Therefore, a module of genes involved in a physiological process may be significantly down-regulated whereas each gene in the module under study may be transcriptionally down regulated by say only 20%, and thus not clearly detected at the individual gene level (Tierney *et al.*, 2003, Wong & Chang, 2005). In addition, beside classical ideas regarding trans-regulation of gene expression, a greater number of hypotheses generated from regulatory networks analysis or cis-regulatory DNA elements analysis can be validated today in a high throughput fashion using chromatin-immunoprecipitation followed by microarray analysis (ChIP-chip). For example, a large fraction of genes transcribed in the liver and pancreas have been found to bind HNF4, providing a molecular explanation for the role of HNF4 mutations and polymorphisms in hereditary and sporadic forms of diabetes mellitus (Odom *et al.*, 2004).

In majority, transcripts are not tissue-restricted, but are present to varying degrees in a wide variety of cell types although there are exceptions, like myosin heavy chain which is primarily found in smooth muscle cells. As such, it is not necessary to create arrays only from cDNAs

obtained from dedicated libraries. Many sources of clones can be used for array analyses including microarrays purchased from companies (e. g. Incyte, Affymetrix, Clontech, etc.), which consist of cDNA clones or oligonucleotides that cover a large percentage of the transcripts (known and ESTs) present in public databases (Juhasz *et al.*, 2002). Only in the context of a well defined cell population, DNA microarray data can be used in a comprehensive analysis aimed at identifying the shared and unique molecular 'modules' underlying a pathological process. Thus, in peripheral organs, laser microdissection of their several constitutive compartments may reveal distinct repertoires of apoptosis-associated genes, chemokines and chemokine receptors in these compartments (Shen *et al.*, 2004).

The microarray technology made possible to widen our comprehension of the major structural and metabolic transformations which affect the myometrium from the very beginning of pregnancy until parturition. The results have proven to be coherent with the mass of information which was accumulated previously, primarily on the basis of the study of selected critical factors. Although still limited in number, the recent studies highlight the activation of precise signaling pathways, some of which may had been under evaluated. Thus, the remodelling and maturation processes that the uterus undergoes in pregnancy appear clearly as phenomenon’s which last during the full course of gestation. This is attested by the nature of best represented signaling pathways, in comparisons of the non pregnant-uterus versus term uterus and the comparisons of the preterm uterus versus the term uterus in labor. Comparatively, the onset of labor is perhaps the phenomenon which remains the least well described by these methods of analysis, possibly because it is a phenomenon occurring in a too short window to have been grasped by the few studies carried out up to now. Whatever it may be, genomic studies constitute a necessary first step of orientation which should lead to a more elaborate hierarchical vision of the physiological mechanisms of gestation, in particular

by establishing new links between the generic signaling pathways that are activated during the normal or pathological gestation. Genomic studies also represent an indicative step that will need to be correlated with a systematic proteomic analysis of the myometrium. The latter will undoubtedly develop in a very near future.

Building comprehensive strategies of genomic and proteomic analysis to explore physiologic functions remains today a challenge to which very few research groups have devoted their energy, and so far only for biological functions unrelated to the pregnant uterus. The examples reported today indicate that this will be hardly accessible without the integrated cooperation of several groups (Ho *et al.*, 2003).

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760

Table 1

Actin cytoskeleton			Focal adhesion			Adherens junctions			Tight junctions		
NP-TNIL[1]	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**
15	6	0	18	6	3	11	3	0	10	0	0
ACTN4(+)	EGF(-)		ACTN4(+)	INSR(+)	ILK(+)	ACTN4(+)	INSR(+)		ACTN4(+)		
CD14(+)	ITGAV(-)		ARHGAP5(+)	ITGB3(-)	THBS1(+)	CTNNA1(+)	PTPRB5(-)		CTNNA1(+)		
EGFR(+)	ITGB3(-)		CAV2(+)	ITGB6(-)	TNC(+)	EGFR(+)	PTPN1(-)		GNAI2(+)		
FGFR1(+)	ITGB6(-)		COL1A2(+)	MAPK10(-)		FGFR1(+)			HCLS1(+)		
HEM1(+)	MATK(-)		EGFR(+)	PIK3CB(-)		NLK(+)			PRKCB1(+)		
ITGAM(+)	PIK3CB(-)		FLNA(+)	RAP1A(-)		PTPN6(+)			PRKCD(+)		
ITGAV(+)			ITGB5(+)			PTPRJ(+)			PRKCG(+)		
ITGB5(+)			PIK3CA(+)			RAC2(+)			RAB13(+)		
PFN1(+)			PIP5K1C(+)			SMAD2(+)			RRAS(+)		
PIK3CA(+)			PRKCB1(+)			TCF7L2(+)			SPTAN1(+)		
PIP5K1C(+)			PRKCG(+)			VCL(+)					
RAC2(+)			ROCK1(+)								
ROCK1(+)			RRAS(+)								
RRAS(+)			TNC(+)								
VCL(+)			VASP(+)								
			VCL(+)								
			VWF(+)								
			ZYX(+)								

Table 1 legend

The main KEGG (http://www.genome.jp/kegg/) metabolic pathways, regulatory pathways or molecular complexes that were found to be the best represented in the myometrium on the basis of genes detected in genomic studies according to [1]NP-TNIL (Rehman *et al.*, 2003), to [2]PT-TNIL (Bethin *et al.*, 2003, Charpigny *et al.*, 2003) or to [3]TNIL-TIL (Esplin *et al.*, 2005, Havelock *et al.*, 2005), during the cycle or pregnancy. All gene

transcripts showed at least a 2 fold or more, up- (+) or down-regulation (-) after paired comparisons of the following stages: non-pregnant (NP) versus term not in labor (TNIL), preterm (PT) versus TNIL, or TNIL versus term in labor (TIL). Number of genes represented in pathways and physiological stages, out of the total number of genes (n=118) sorted as belonging to these pathways, “0” means no gene represented for the corresponding pathway. OMIM nomenclature adopted for gene names (http://www.ncbi.nlm.nih.gov/omim/).

Table 2

Cytokine-cytokine receptors			Toll-like receptors			TGF-beta signaling pathway			Wnt signaling pathway		
NP-TNIL[1]	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**
8	12	5	5	6	1	6	2	3	7	2	1
CCL19(+)	BLR1(+)	CCL13(+)	CD14(+)	IKBKB(-)	CXCL10(+)	ACVRL1(+)	THBS2(+)	TGFB2(+)	NLK(+)	MAPK10(-)	VANGL1(+)
CCL21(+)	CCL13(+)	CCL18(+)	IL6(+)	IRAK1(-)		BMP6(+)	TNF(-)	THBS1(+)	PRKCB1(+)	TP53(+)	
CSF1R(+)	CXCR4(+)	CXCL10(+)	PIK3CA(+)	MAP2K4(-)		ROCK1(+)		THBS2(+)	PRKCG(+)		
EGFR(+)	IL9(+)	TGFB2(+)	RAC2(+)	MAPK10(-)		SMAD2(+)			RAC2(+)		
IL15RA(+)	TNFRSF6(+)	TNFRSF11B(+)	STAT1(+)	PIK3CB(-)		TFDP1(+)			ROCK1(+)		
IL6(+)	CLC(-)			TNF(-)		THBS2(+)			SMAD2(+)		
IL6ST(+)	EGF(-)								TCF7L2(+)		
TNFRSF11B(+)	IFNGR1(-)										
	IL6ST(-)										
	LIFR(-)										
	TNF(-)										
	TNFSF4(-)										

Table 2 legend

See Table 1 legend

Table 3

MAPK signaling pathway			Phosphatidylinositol signaling pathway			JAK-STAT signaling pathway		
NP-TNIL[1]	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**
15	12	4	5	8	0	6	7	1
CD14(+)	EGF(+)	BDNF(+)	IMPA2(+)	CALM1(-)		IL15RA(+)	IL9(+)	IL13(+)
EGFR(+)	IKBKB(+)	DUSP5(+)	PIK3CA(+)	CALM3(-)		IL6(+)	PIM1(+)	
FGFR1(+)	MAP2K4(+)	NR4A1(+)	PIP5K1C(+)	DGKZ(-)		IL6ST(+)	CLC(-)	
FLNA(+)	MAPK10(+)	TGFB2(+)	PRKCB1(+)	ITPR1(-)		PIK3CA(+)	IFNGR1(-)	
HSPA5(+)	PLA2G5(+)		PRKCG(+)	ITPR1(-)		PTPN6(+)	IL6ST(-)	
HSPA8(+)	RAP1A(+)			PIK3CB(-)		STAT1(+)	LIFR(-)	
MAP3K5(+)	RASA1(+)			PLCG1(-)			PIK3CB(-)	
NLK(+)	TNF(+)			PLCG2(-)				
PLA2G2A(+)	TP53(+)							
PRKCB1(+)	PLA2G2A(-)							
PRKCG(+)	PPP5C(-)							
RAC2(+)	TNFRSF6(-)							
RASGRP2(+)								
RRAS(+)								
YWHAZ(+)								

Table 3 legend

See Table 1 legend

Table 4

Apoptosis			**Calcium signaling pathway**			**Neuroactive ligand-receptors**		
NP-TNIL[1]	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**	**NP-TNIL[1]**	**PT-TNIL[2]**	**TNIL-TIL[3]**
0	6	0	8	8	0	6	6	0
	TNFRSF6(+)		EGFR(+)	BST1(+)		ADORA3(+)	GLRB(+)	
	TP53(+)		GNAQ(+)	CALM1(-)		ADRA2C(+)	GABRB1(-)	
	DFFA(-)		LYN(+)	CALM3(-)		C3AR1(+)	GABRG2(-)	
	IRAK1(-)		NFKBIB(+)	ITPR1(-)		EDN1(+)	PTGER3(-)	
	PIK3CB(-)		PHKA1(+)	PLCG1(-)		GRM2(+)	TACR2(-)	
	TNF(-)		PRKCB1(+)	PLCG2(-)		NPY(+)	TRHR(-)	
			PRKCG(+)	PTGER3(-)				
			PYGL(+)	TACR2(-)				

Table 4 legend

See Table 1 legend

Figure legends

Figure 1

Main KEGG pathways (http://www.genome.jp/kegg/) represented in the myometrium on the basis of the genes detected in genomic studies. Gene transcripts pertaining to these pathways were either up-regulated (red) or down-regulated (green) by a two fold or more factor. White boxes denote insufficiently documented links in context. See Tables 1-4 for details. (A) Comparison between the non-pregnant (NP) and term not in labor (TNIL) uterus (Rehman et al., 2003). (B) Comparison between the preterm (PT) uterus and term in labor uterus (TNIL) (Bethin et al., 2003, Charpigny et al., 2003) or between TNIL and term in labor uterus (TIL) (Esplin et al., 2005, Havelock et al., 2005). Five pathways (Focal adhesion, MAPK, TGF-beta, JAK-STAT and apoptosis) are shown with their known links to membrane receptors (Wnt, TGF-beta, cytokines and Toll-like receptors). Three additional pathways (actin cytoskeleton, phosphatidylinositol and calcium signaling are over-expressed in gestation by comparison to the non-pregnant state. THBS1, thrombospondin-1 (inhibitory).

Figure 2

Main KEGG pathways (http://www.genome.jp/kegg/) represented in the myometrium on the basis of the genes detected in genomic studies. Gene transcripts pertaining to these pathways were either up-regulated (red) or down-regulated (green) by a two fold or more factor. White boxes denote insufficiently documented links in context. See Tables 1-4 for details. (A) Comparison between the non-pregnant (NP) and term not in labour (TNIL) uterus (Rehman et al., 2003). (B) Comparison between the preterm (PT) uterus and term in labor uterus

(TNIL) (Bethin et al., 2003, Charpigny et al., 2003) or between TNIL and term in labor uterus (TIL) (Esplin et al., 2005, Havelock et al., 2005). Ten pathways (focal adhesion, adherens junctions, tight junctions, actin cytoskeleton, phosphatidylinositol and calcium signaling, MAPK, TGF-beta, JAK-STAT and apoptosis) are shown with their known links to cytokine, growth factors (GF) and G-protein coupled membrane receptors. CAMs, cell adhesion molecules, ECM, extra-cellular matrix.

Figure 1

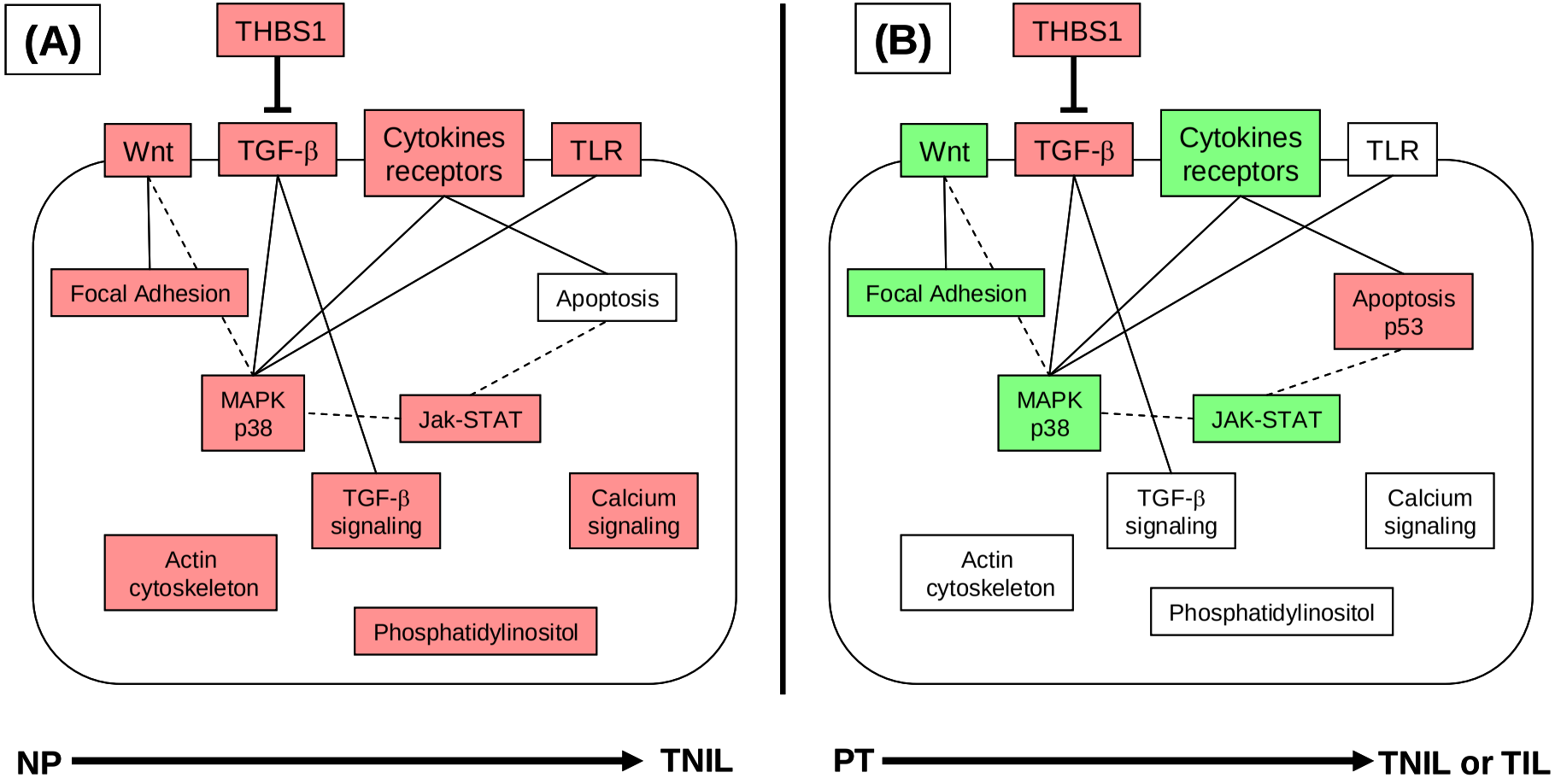

Figure 2