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1 **Gene and protein expression in myometrium in pregnancy and labor**

2

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4

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10 **Abstract**

11

12 Microarray technologies widen our comprehension of the major structural and metabolic
13 transformations which affect the myometrium from the very beginning of pregnancy until
14 parturition. The results are coherent with the mass of information which was accumulated
15 previously, primarily on the basis of studies of selected critical factors. They highlight the
16 activation of precise signaling pathways, some of which may have been previously under
17 evaluated. The remodelling and maturation processes that the myometrium undergoes in
18 pregnancy appear clearly as phenomenon's which last during the full course of gestation.

19 Comparatively, the onset of labor is perhaps the phenomenon which remains the least well
20 described by these methods of analysis. Nevertheless, genomic studies constitute a necessary
21 first step of orientation and help establishing new links between the generic signaling
22 pathways that are activated during the normal or pathological gestation. These studies also
23 represent an indicative step that will have to be paralleled, in the future, with the results of the
24 systematic proteomic analysis of the myometrium.

25 **Introduction**

26

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

34

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

44

45 **Functional genomic studies during pregnancy**

46

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

50 *al.*, 2003, Marvin *et al.*, 2002a, 2002b, Tashima *et al.*, 2002), and different tissues:
51 myometrium (Aguan *et al.*, 2000, Bethin *et al.*, 2003, Chan *et al.*, 2002, Charpigny *et al.*,
52 2003, Esplin *et al.*, 2005, Havelock *et al.*, 2005, Salomonis *et al.*, 2005), fetal membranes
53 (Chen *et al.*, 2002, Keelan *et al.*, 2003, Marvin *et al.*, 2002a, 2002b, Muhle *et al.*, 2001,
54 Tashima *et al.*, 2002), trophoblast (Chen *et al.*, 2002, Kato *et al.*, 2002). Various
55 methodologies have been used: Suppression Subtractive Hybridization (SSH) (Chan *et al.*,
56 2002) or microarrays for most of the previous cited studies. All investigators have reported
57 genes differentially regulated during parturition (Romero *et al.*, 2002) and multiple novel
58 candidate markers for preterm labor (Chan *et al.*, 2002, Marvin *et al.*, 2002a, 2002b, Wu *et*
59 *al.*, 1999) or for premature rupture of membranes (Tashima *et al.*, 2002). They contributed to
60 shed light on specific pathophysiological issues like the patterns of expression of cytokines in
61 the fetal membranes and decidua particularly in the presence of intrauterine infection (Keelan
62 *et al.*, 2003) or on the induction of enzymes for prostaglandin synthesis (Bethin *et al.*, 2003).
63 Groups of genes with yet unknown functional connection to these pathologies were also
64 found coordinately expressed. This was emphasized in animal studies which allow to explore
65 the global pattern of gene expression and their co-regulation on the basis of their genomic
66 location over the full time-course of myometrial transformation (i.e. pregnancy) or following
67 experimentally-controlled infection (Girotti & Zingg, 2003, Muhle *et al.*, 2001, Salomonis *et*
68 *al.*, 2005, Wu *et al.*, 1999).

69
70 Computational methods have significantly helped to the interpretation of gene profiling
71 experiments by delineating clusters of genes sharing coherent expression features (Claverie,
72 1999). During the last five years, statistical methods and data analysis for array studies have
73 progressed enormously and today an “ideal” study should now incorporate i) careful research
74 designs (differential expression or cross sectional studies), ii) statistical methods

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

83
84 The biases adopted in this review are several. We focused attention on genes expressed
85 essentially in the “normal” human myometrium. One study has reported a direct comparison
86 of the non-pregnant myometrium (NP) with the pregnant human myometrium at term not in
87 labor (TNIL) (Rehman *et al.*, 2003). It gives an overall picture of the changes that the uterine
88 muscle undergoes in its adaptation to the gestation. Two studies have described the changes in
89 gene expression in the myometrium in preterm patients not in labor (PT) versus patients at
90 term not in labor (TNIL) (Bethin *et al.*, 2003, Charpigny *et al.*, 2003) and two additional
91 studies have described the changes in gene expression in the myometrium in patients at term
92 not in labor (TNIL) versus patients at term in labor (TIL) (Esplin *et al.*, 2005, Havelock *et al.*,
93 2005). Other studies have not been compiled directly, either because they did not provide
94 sufficient precise references on the genes being studied (i.e. lack of GenBank or Unigene
95 references), they considered other components of the utero-fetal unit (i.e. membranes,
96 decidua, cervix, etc.) or they were carried out on animal species. To translate lists of tens or
97 hundreds of genes found to be differentially regulated in the conditions under study into a
98 clearer understanding of the biological phenomena involved, we used both combinations of
99 searches through the literature referenced in public databases and the Onto-Tools software

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

110

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

119

120 **Changes in structural and contractile genes during gestation**

121

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

125

126 ***Regulation of actin cytoskeleton***

127

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

139

140 RhoA, a member of the Ras superfamily, and its downstream mediator RhoA kinase
141 (ROCK1) are necessary for agonist-induced stress fiber formation (Gogarten *et al.*, 2001).

142 The higher expression of protein kinase C (PKC) isoforms observed in pregnant *versus* non-
143 pregnant myometrium (Rehman *et al.*, 2003) may promote or drive the formation of stress
144 fibers. Additionally, up-regulation of ROCK1 has been demonstrated in human term
145 myometrium (Moore *et al.*, 2000, Rehman *et al.*, 2003). This leads to the idea that increased
146 endogenous ROCK1 activity, resulting in enhanced RhoA-mediated calcium sensitization, is
147 involved in the increased contractility at the time of labor. It is of note that EGF increases the
148 presence of actin and myometrial EGF receptors transcripts are increased at the time of
149 parturition (Charpigny *et al.*, 2003, Gargiulo *et al.*, 1997, Rehman *et al.*, 2003).

150

151 ***Focal adhesion***

152

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

160

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

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

178

179 **Parturition as an inflammatory process**

180

181 *Cytokine-chemokines interaction*

182

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

187

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

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

202
203 The TNF receptor super family member Fas and its cognate ligand (FasL) play a role in cell
204 leiomyoma apoptosis (Wang Y. *et al.*, 2002). Therefore increased expression of TNFRSF6 (i.e.
205 Fas receptor) in term myometrium (Table 2) await consideration in the control of the uterine
206 growth process. Elevated expression of TNFRSF11B is observed in human myometrium with
207 or without labor (Esplin *et al.*, 2005, Rehman *et al.*, 2003). A peak in uterine osteoprotegerin
208 (i.e OPG or TNFRSF11B), a soluble membrane of the TNF-alpha receptor family that acts as a
209 negative regulator for receptor activator of nuclear factor-kB (RANK), has been reported
210 during labor in the rat (Girotti & Zingg, 2003).

211
212 The gp130 protein is a subunit component of several cytokines receptors including those for
213 LIF. Cytokines sharing the gp130 (or IL6ST) subunits are referred to as IL6 type family of
214 cytokines and signal through JAK/STAT pathway (Heinrich *et al.*, 1998). They play a role in
215 the regulation of gene activation, proliferation and differentiation. Down-regulation of IL6ST
216 and LIF receptor at term (Table 3) may be an indication of the decrease of proliferation
217 processes before the onset of labor.

218
219 IFN-gamma inhibits the proliferation of vascular smooth muscle cells and the synthesis of
220 collagens by myofibroblasts. In human myometrial cells, interferon gamma antagonizes IL-
221 1beta-induced prostaglandin-endoperoxide synthase 2 (COX-2 or PTGS2) expression and
222 PGE2 production (Hertelendy *et al.*, 2002). Therefore, the decrease in IFNGR1 transcript
223 observed during the transition from PT to TNIL states of the uterus (Table 2), could be
224 interpreted as an *ad hoc* evolution for labor onset.

225

226 ***TLR signaling pathway***

227

228 Toll-like receptors (TLRs) are evolutionarily conserved pathogen-associated microbial
229 patterns and play important roles in innate immunity in mammals. Ten TLRs are evidenced in
230 the uterus but TLR2 and TLR4 mRNA are expressed at the highest levels. Among the TLR-
231 related gene transcripts, SOCS, CD14 and IRAK mRNAs (see Tables 2 and 4) are widely
232 expressed in the myometrium (Nishimura & Naito, 2005). TLR4 mediates induction of PTL
233 in mice treated with lipopolysaccharide (LPS) (Wang H. & Hirsch, 2003). In monocytes,
234 LPS-induced signaling through TLRs, lead to the recruitment of docking proteins such as IL-1
235 receptor-associated kinase (IRAK) and TNF-alpha receptor-associated factor (TRAF6)
236 leading to the activation of IkappaB kinase (IKK) complex. These pathways in turn activate
237 transcription factor such as NF-kB that coordinates the induction of genes encoding
238 inflammatory mediators (Guha & Mackman, 2001). In human pregnant myometrial cells a
239 positive immunoreactivity for TLR4 is observed and cells exposure to LPS lead to nuclear
240 translocation of the p65 subunit of NFkB (Dallot *et al.*, 2005). The increased expression of
241 CD14 together with increased expression of the catalytic subunit of PI3K in pregnant
242 myometrium may serve to increase host protection against microbial invasion (Rehman *et al.*,
243 2003). At the end of pregnancy, because some of the TLR-related genes such as IRAK, IKK,
244 MKK have decreased expression compared to earlier stage of the gestation (Charpigny *et al.*,
245 2003), it is possible that adaptative responses to immune or growth processes take place.

246

247 ***TGF-beta signaling pathway***

248

249 TGF-beta regulates its own expression, the expression of ECM, of matrix metalloproteinases
250 (MMP), and of tissue inhibitor of matrix metalloproteinases (TIMP) as well as the growth of
251 leiomyomas and of normal myometrial cells (Chegini *et al.*, 1999). TGF-beta receptors
252 mediate their action through multiple pathways, including smad proteins that convey TGF-
253 beta receptor signals from the cell surface to the nucleus, resulting in transcriptional activation
254 of TGF-beta-responsive genes. TGF-beta also activates mitogen-activated protein kinase
255 (MAPK), PKC, and calcium/calmodulin complex, inducing Smad-independent transcriptional
256 responses. One mechanism by which TGF-beta is activated involves thrombospondin
257 (THBS1). THBS1 promotes replication of smooth cells and modifies responses to contractile
258 agents. THBS1 expression and protein in human myometrial tissues are increased during
259 labor and after the administration of OT (Morimoto *et al.*, 1998). Myometrial transcripts of
260 THBS1 and THBS2 increases during pregnancy (Charpigny *et al.*, 2003, Rehman *et al.*, 2003)
261 and peak at the time of labor concomitantly with the expression of TGF-beta (Esplin *et al.*,
262 2005). It is possible that the control of the TGF-beta pathway in the near term myometrium
263 facilitates the transition of the quiescent uterus towards a contractile state, as well as it
264 contributes to control the uterine growth.

265

266 ***Wnt signalling pathway***

267

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

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

276

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

279

280 ***MAPK-signaling pathway***

281

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

297

298 In the myometrium, the MAPK pathways are also implicated in the induction of PTGS2
299 expression (Bartlett *et al.*, 1999, Sooranna *et al.*, 2004) and in mediating the effects of
300 prostaglandins (PGs) (Ohmichi *et al.*, 1997), OT (Zhong *et al.*, 2003), endothelin-1 (ET-1)
301 (Kimura *et al.*, 1999) and corticotropin-releasing hormone (CRH) (Grammatopoulos *et al.*,
302 2000, Papadopoulou *et al.*, 2004). Increasing maternal plasma levels of CRH during the last
303 weeks of pregnancy and the substantial expression of CRH receptors in choriodecidua,
304 placenta and myometrium suggests that this stress hormone plays a role in the control of
305 human parturition (Sehringer *et al.*, 2004).

306

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

315

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

322 for IGF-1 induction of uterine nuclear proliferative responses, and a cross-talk between IGF-
323 1R and ER signaling pathways exists *in vivo* (Klotz *et al.*, 2002).

324

325 ***Protein kinase C***

326

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

334

335 A balance between T helper (Th1) (pro-inflammatory) / Th2 (anti-inflammatory) cytokine
336 production, has been described at the time of parturition, and a link between TNF-
337 alpha/IL1-beta and premature human childbirth has been proposed (Arntzen *et al.*, 1998).
338 Because, atypical PKC's are important components of the TNF/IL1B signaling pathway that
339 controls NF- κ B activation, the implication of the PRKCZ in the control of the onset of
340 labor in women is not surprising. (Ozaki *et al.*, 2003) also demonstrated that the levels of
341 mRNA of PRKCB1 isoform in pregnant human myometrium were greater than those in
342 nonpregnant myometrium, a feature confirmed in genomic studies (Table 3) (Rehman *et al.*,
343 2003). The levels of the phosphorylated substrate for PKC, CPI-17 which is considered to
344 inhibit myosin light chain phosphatase were also greater in the pregnant myometrium. These
345 results suggest that the PKC-mediated contractile mechanism is augmented in human

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

348

349 ***Phosphatidyl inositol signaling pathway***

350

351 The PI3-kinase cascade is activated through the binding of the regulatory subunit to
352 phosphorylated tyrosine residues, leading to enhanced activity of the catalytic subunit. PI3
353 kinase phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) to generate PIP3 which
354 interacts with protein kinase Akt or protein kinase B (PKB). Activation of Akt influences
355 many cellular functions through PIP3 binding and phosphorylation by phosphoinositide-
356 dependent kinase 1 (PDK1). It includes cytoskeletal organization, cell growth, motility,
357 proliferation and survival. PDK1 is known to phosphorylate PKC-zeta in the activation loop.
358 PI3 kinase is also activated by direct binding of the catalytic subunit to activated Ras and PI3
359 kinase cascade activation can lead to the activation of the ERK cascade (Stein & Waterfield,
360 2000).

361

362 Increases in $[Ca^{2+}]_i$ are controlled by multiple signal pathways in myometrium. GPCR-
363 mediated stimulation of the Gq/11 subfamily and subsequent activation of PLC subfamily
364 results in generation of IP3, which triggers release of Ca^{2+} from intracellular stores.

365 Five PLC isoforms: beta 1, beta 2, beta 3, gamma 1, and gamma 2 are detected in human
366 myometrium. OT activates human myometrium by interacting with at least two G proteins
367 and possibly three PLC beta isoforms (Phaneuf *et al.*, 1996). Indeed, the amount of PLCB4 is
368 increased at midpregnancy, whereas PLCB1, PLCB2, and PLCB3 are up-regulated at term in
369 the rat uterus (Mhaouty-Kodja *et al.*, 2004). PLCB3 may be targeted by both contractant and
370 relaxant signaling pathways in myometrium and play a critical role in the balance between

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

376

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

383

384 **Apoptosis and parturition**

385

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

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

398

399 **G-protein signaling and parturition**

400

401 The silencing of the myometrial contractile function is conditioned by a predominant
402 functional cAMP/cGMP system, whereas contractions are under the control of agonist-
403 induced calcium mobilization via the PLC pathway. Gestational-related modifications of
404 GPCR in the myometrium, as well as changes in their associated kinases (GRK), cognate G
405 proteins, and effectors have been detected (Hertelendy & Zakar, 2004, Lopez Bernal &
406 TambyRaja, 2000). Some GPCRs coupled to PLC generates the second messengers IP3 and
407 DAG. The ensuing rise in IP3 releases Ca²⁺ from the sarcoplasmic reticulum, causing a
408 sudden rise in intracellular calcium, whereas DAG activates PKC and the MAPK cascades.
409 Uterine contractility can also be enhanced via the activation of Rho GTPases, and the
410 subsequent action of ROCK's to potentiate the effect of myosin light chain kinase (MYLK).
411 These pathways have been discussed above. Other GPCR coupled to the adenylyl cyclase
412 promotes the accumulation of cAMP which controls the relaxation of the myometrium during
413 pregnancy via the inactivation of MYLK and the activation of ATF-2 (Bailey & Europe-
414 Finner, 2005). Another powerful way for increasing cAMP concentration consists in
415 inhibiting its hydrolysis by phosphodiesterases (PDEs). Among the five PDE families (PDE 1,
416 2, 3, 4 and 5) identified in the human myometrium (Leroy *et al.*, 1994, Leroy *et al.*, 1999),
417 one particular isoform, PDE4B2, which specifically hydrolyses cAMP is selectively induced
418 at the end of pregnancy suggesting a role for this protein in the setting of the contractile state
419 of the myometrium just before delivery (Mehats *et al.*, 2001, Mehats *et al.*, 2000).

420

421 **Neuroactive-ligands and parturition**

422

423 A small selection of peptides, neuroactive factors and ion channels such as ET-1,
424 noradrenaline, NPY, PGE2, GABA... that mediate uterine relaxation or contraction and act
425 through GPCR are mentioned here (see Table 4). ET-1 exerts a stimulatory effect on
426 myometrium contractility and proliferation. Its transcript is up-regulated in the pregnant
427 myometrium (Rehman *et al.*, 2003). Among the three subtypes of alpha2-adrenoceptors found
428 in the human myometrium at term pregnancy (Adolfsson *et al.*, 1998), only the alpha2C-
429 subtype appears to be predominant in pregnant myometrium. Stimulation of GABA (A)
430 receptors tonically inhibits contractions of the rabbit myometrium, while stimulation of
431 GABA (B) receptors enhances contractions. Steroids interact with GABA (A) receptors to
432 modulate uterine contractility (Majewska & Vaupel, 1991). The subunit composition of
433 GABA (A) receptor differs in rat uteri throughout gestation and just before labour in humans,
434 a decline in GABA receptor transcripts is observed (Bethin *et al.*, 2003, Charpigny *et al.*,
435 2003). TRH-R is expressed in term myometrium. (Fukusumi *et al.*, 1995) have previously
436 reported high level of TRH-R mRNA in uterus but whether TRH plays an important role in
437 the female reproductive tract remains to be elucidated. PGs, in conjunction with their
438 numerous receptors activate multiple signaling pathways and exert multifaceted actions in
439 myometrium (Hertelendy & Zakar, 2004). Thus, EP3 transcripts are down-regulated in the
440 term pregnant myometrium (Bethin *et al.*, 2003, Charpigny *et al.*, 2003). However, the
441 significance of these changes remains to be established, because of the known existence of
442 EP3 splice variants.

443

444 **Future strategies for integrative analysis of myometrial functions**

445

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

452
453 Gene module analysis, as exemplified in this review, searches for coordinate regulation of
454 genes that belong to a priori defined gene modules. A statistical test performed for each
455 module relative to all other genes on the microarray calculates whether the degree of
456 coordinate regulation is more than one would expect by chance. Therefore, a module of genes
457 involved in a physiological process may be significantly down-regulated whereas each gene
458 in the module under study may be transcriptionally down regulated by say only 20%, and thus
459 not clearly detected at the individual gene level (Tierney *et al.*, 2003, Wong & Chang, 2005).
460 In addition, beside classical ideas regarding trans-regulation of gene expression, a greater
461 number of hypotheses generated from regulatory networks analysis or cis-regulatory DNA
462 elements analysis can be validated today in a high throughput fashion using chromatin-
463 immunoprecipitation followed by microarray analysis (ChIP-chip). For example, a large
464 fraction of genes transcribed in the liver and pancreas have been found to bind HNF4,
465 providing a molecular explanation for the role of HNF4 mutations and polymorphisms in
466 hereditary and sporadic forms of diabetes mellitus (Odom *et al.*, 2004).

467
468 In majority, transcripts are not tissue-restricted, but are present to varying degrees in a wide
469 variety of cell types although there are exceptions, like myosin heavy chain which is primarily
470 found in smooth muscle cells. As such, it is not necessary to create arrays only from cDNAs

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

480

481 The microarray technology made possible to widen our comprehension of the major structural
482 and metabolic transformations which affect the myometrium from the very beginning of
483 pregnancy until parturition. The results have proven to be coherent with the mass of
484 information which was accumulated previously, primarily on the basis of the study of selected
485 critical factors. Although still limited in number, the recent studies highlight the activation of
486 precise signaling pathways, some of which may had been under evaluated. Thus, the
487 remodelling and maturation processes that the uterus undergoes in pregnancy appear clearly
488 as phenomenon's which last during the full course of gestation. This is attested by the nature
489 of best represented signaling pathways, in comparisons of the non pregnant-uterus versus
490 term uterus and the comparisons of the preterm uterus versus the term uterus in labor.

491 Comparatively, the onset of labor is perhaps the phenomenon which remains the least well
492 described by these methods of analysis, possibly because it is a phenomenon occurring in a
493 too short window to have been grasped by the few studies carried out up to now. Whatever it
494 may be, genomic studies constitute a necessary first step of orientation which should lead to a
495 more elaborate hierarchical vision of the physiological mechanisms of gestation, in particular

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

500
501 Building comprehensive strategies of genomic and proteomic analysis to explore physiologic
502 functions remains today a challenge to which very few research groups have devoted their
503 energy, and so far only for biological functions unrelated to the pregnant uterus. The
504 examples reported today indicate that this will be hardly accessible without the integrated
505 cooperation of several groups (Ho *et al.*, 2003).

506

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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 ¹NP-TNIL (Rehman *et al.*, 2003), to ²PT-TNIL (Bethin *et al.*, 2003, Charpigny *et al.*, 2003) or to ³TNIL-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(-)		TFDPI(+)			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	TNIL-TIL ³
0	6	0	8	8	0	6	6	0	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

1 **Figure legends**

2

3 **Figure 1**

4

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

17

18 **Figure 2**

19

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

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

32

Figure 1

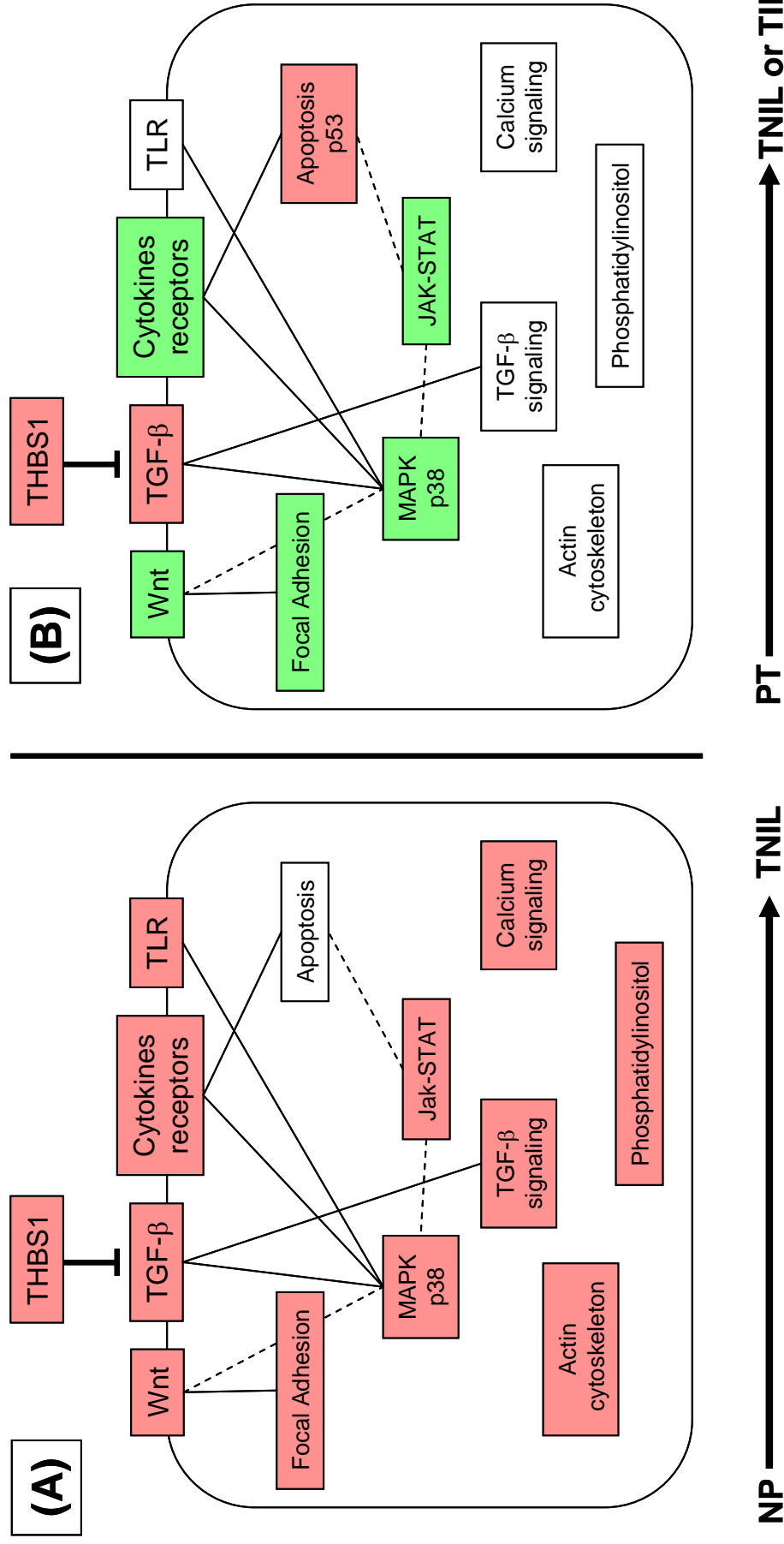


Figure 2

