

—Review—

Regulation of mitogen-activated protein kinase 3/1 activity during meiosis resumption in mammals

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Abstract. *In vivo*, resumption of oocyte meiosis occurs in large ovarian follicles after the preovulatory surge of luteinizing hormone (LH). The LH surge leads to the activation of a broad signaling network in mural granulosa cells equipped with LH receptors. The signals generated in the mural granulosa cells are further augmented by locally produced peptides or steroids and transferred to the cumulus cell compartment and the oocyte itself. Over the last decade, essential progress has been made in the identification of molecular events associated with the final maturation and ovulation of mammalian oocytes. All new evidence argues for a multiple roles of mitogen-activated protein kinase 3/1 (MAPK3/1) in the gonadotropin-induced ovulation processes. However, the knowledge of gonadotropin-induced signaling pathways leading to MAPK3/1 activation in follicular cells seems limited. To date, only the LH-induced transactivation of the epidermal growth factor receptor/MAPK3/1 pathway has been described in granulosa/cumulus cells even though other mechanisms of MAPK3/1 activation have been detected in other types of cells. In this review, we aimed to summarize recent advances in the elucidation of gonadotropin-induced mechanisms leading to the activation of MAPK3/1 in preovulatory follicles and cultured cumulus-oocyte complexes and to point out a specific role of this kinase in the processes accompanying final maturation of the mammalian oocyte.

Key words: Cumulus-oocyte complexes, Meiosis resumption, Mitogen-activated protein kinase 3/1 (MAPK3/1), Preovulatory follicle

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During the growth phase, the mammalian oocyte is arrested in the first meiotic prophase, but it gains full meiotic competence in several steps [1]. However, these meiotically competent oocytes still remain in the dictyate stage if they are retained within follicles. *In vivo*, resumption of meiosis occurs in large ovarian follicles after the preovulatory surge of luteinizing hormone (LH). This LH surge leads to the activation of a broad signaling network in mural granulosa cells equipped with LH receptors. The signals generated in the mural granulosa cells are further augmented by locally produced peptides or steroids and transferred to the cumulus cell compartment and the oocyte itself. Complex interaction and feedback loops among all these ovarian cell types result in the resumption of meiosis in oocytes, expansion of surrounding cumulus cells, ovulation of the matured oocyte-cumulus complex into the oviduct and luteinization of the granulosa cells. Over the last decade, substantial progress has been made in identifying molecular events associated with the final maturation and ovulation of mammalian oocytes. The aim of this review was to summarize the mechanisms of the gonadotropin-induced activation of mitogen-activated protein kinase (MAPK) 3/1 in preovulatory follicles and in cultured cumulus-oocyte complexes (COCs) in rodents and domestic animal species. Special attention was paid to the role of MAPK3/1 in meiotic resumption and the

accompanying events. The role of MAPK3/1 in regulation of granulosa cell proliferation and survival and in postovulatory events was described elsewhere [2–4].

Role of MAPK3/1 in Oocytes

MAPK3/1, commonly also known as extracellular-regulated protein kinase (Erk1 and Erk2), is activated by MEK1/2 through phosphorylation at the threonine 183 and tyrosine 185 residues. MEK1/2 is also activated by phosphorylation at serine 217 and 221; direct upstream activators of this kinase are Raf proteins (A-Raf, B-Raf or c-Raf). In vertebrate oocytes, MEK is activated by the protein c-Mos [5, 6].

MAPK3/1 is an abundant protein in vertebrate oocytes, and its relationship with regulation of the meiotic cell cycle has been extensively studied. In *Xenopus* oocytes, the induction of germinal vesicle breakdown (GVBD) by progesterone, androgen or growth factors requires the activation of MAPK3/1. It is thought that the external stimuli induce the expression of c-Mos mRNA (hormones) or activate Ras/Raf (growth factors) and thus trigger the MAPK3/1 signaling cascade. The activated MAPK3/1 then affects meiotic resumption by removing the inhibitory influence of Myt1 kinase on maturation promoting factor (MPF) and by a positive feedback loop increasing the synthesis of Mos [7, 8]. In mammalian oocytes, the activation of MAPK3/1 occurs at the time of or even hours after GVBD [9–14]. In addition, a previous study reported that GVBD occurred in c-Mos knockout mice that lack the ability to activate MAPK3/1 [15]. Bovine oocytes injected with MKP1 mRNA, which encodes a dual-specificity MAPK phosphatase, resumed and progressed

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through meiosis, despite the lack of MAPK activity [16]. Inhibition of MEK by U0126 as well as injection of c-mos antisense did not prevent GVBD in denuded pig oocytes [17, 18]. All this evidence suggests that oocyte MAPK3/1 is not involved in the regulation of meiotic resumption in mammals. Nevertheless, MAPK3/1 plays an important role in organizing the spindle, metaphase I/metaphase II transition and metaphase II arrest in mammalian oocytes [16, 19, 20].

Activation of MAPK3/1 in Cumulus Cells is Necessary for the Resumption of Meiosis in the Oocyte

It has been shown in the mouse model that MAPK3/1 activity in cumulus cells is required for gonadotropin-induced GVBD and for induction of cumulus expansion [21]. MAPK3/1 in cumulus cells was activated before GVBD both *in vivo* and *in vitro*, and pharmacological inhibition of MAPK3/1 activation with the MEK inhibitor blocked FSH-induced GVBD *in vitro*. Since spontaneous, gonadotropin-independent GVBD was not blocked by MAPK3/1 inhibition and *Mos*^{-/-} oocytes do not exhibit MAPK3/1 activity, even in the absence of a MEK inhibitor, it follows that the MEK inhibitor must target MAPK3/1 activity induced by FSH in the cumulus cell compartment [21]. Moreover, it has been demonstrated that MAPK3/1 activity in cumulus cells is also required for gonadotropin-induced cumulus expansion and enhanced expression of expansion-related genes (*Has2* and *Ptgs2*) and that this MAPK3/1-dependent step occurs downstream of the cAMP production and activation of protein kinase A (PKA) [21, 22]. Pharmacological disruption of MAPK3/1 signaling by administration of a MEK inhibitor to eCG/hCG-primed mice resulted in inhibition of oocyte maturation, cumulus expansion and follicular rupture and was underlined by a substantially reduced expression of genes involved in these processes [23]. In pigs, the activation of MAPK3/1 in cumulus cells is also a prerequisite for gonadotropin-stimulated resumption of meiosis in cumulus-enclosed oocytes [14, 24, 25]. We recently showed that both gonadotropin- and amphiregulin-induced meiotic resumption depend on MAPK3/1 activity in cumulus cells [26, 27]. The activation of MAPK3/1 was detected in pig cumulus cells 0.5–1 h after the addition of FSH/LH [28–30], and the high activity persisted after at least 32 h of culture [31]. The phosphorylation levels of MAPK3/1 may be affected by culture conditions and may differ from those occurring *in vivo*, as documented in bovine cumulus cells [32].

Mechanisms by which MAPK3/1 Regulates Meiotic Resumption

MAPK3/1 may regulate the events accompanying meiotic resumption by activation of various transcription factors or by a posttranscriptional mechanism based on the phosphorylation of specific proteins in cumulus cells or oocytes. In mouse cumulus/granulosa cells, the transcription factor *C/EBPβ* seems to be one of the major targets of MAPK3/1, since disruption of the *Cebpb* gene in granulosa cells produced a similar phenotype, as seen in MAPK3/1-deficient mice [33]. A detailed study on the roles of *C/EBPα* and *C/EBPβ* in regulation of ovulatory events showed that these transcription factors are critical for follicular rupture and luteinization, but *Cebpa/b*^{gc-/-} granulosa cell-specific knockout mice,

unlike *Erk1/2*^{gc-/-} mice, did not exhibit major defects in LH-induced oocyte maturation and cumulus expansion [34]. A comparison of the granulosa cell transcriptomes of *Cebpa/b*^{gc-/-} and *Erk1/2*^{gc-/-} mice at 4 h post hCG administration revealed that only a subset of genes regulated by *Erk1/2* (~19%) was under the control of *C/EBPα/β* [34]. This indicates that other transcription factors mediate MAPK3/1 action in the preovulatory follicle. The transcription factor *EGR1* is one of the hot candidates since its expression was suppressed in mice with disrupted MAPK3/1 signaling and silencing of *Egr1* in the granulosa cell reduced expression levels of *Ptgs2*, a key gene involved in follicular rupture [23].

Several potential mechanisms have been proposed to explain how the activation of MAPK3/1 in cumulus cells affects the resumption of meiosis in the oocyte. First, the MAPK3/1 pathway may stimulate the synthesis of meiosis-activating molecules, for example, steroids (MAS), that are transported via gap junctions to oocytes [35]. Second, it has been shown that inhibition of the MAPK3/1 pathway in cultured mouse follicles by U0126 blocks LH-induced gap junction closure and oocyte maturation, indicating that activated MAPK3/1 mediates LH-induced meiotic resumption by interrupting cell-to-cell communication within the follicle, possibly through the phosphorylation of connexin 43 [36]. It is important to note that these meiosis-activating events occurred in the follicles within minutes after LH addition. Further experiments indicated that LH-induced gap junction closure is also regulated by other signaling pathways, such as p38MAPK [37]. The existence of a redundant pathway that functions in parallel to the MAPK3/1-dependent closure of connexin 43 gap junctions, was reported to be essential for LH-induced meiotic resumption in mice [38]. The role of gap junction permeability in meiotic resumption in domestic animal species is still unclear. Surprisingly, a previous study showed that gap junction network expansion in pig cultured COCs increased in a gonadotropin-independent manner during the first 8.5 h, but gonadotropin-dependent closure of gap junctions occurred after 6.5 h of culture [39]. Sasseville *et al.* [40] also reported an initial gonadotropin-independent increase in gap junctional communication in cultured pig COCs, which persisted for the first 18 h of culture. A gonadotropin-dependent breakdown of gap junctional communication occurred thereafter, simultaneously with GVBD. Similarly, Isobe *et al.* [41] found in pig COCs that disruption of gap junction communication between cumulus cells gradually increased beyond 8 h of culture and was positively correlated with oocyte GVBD. The possible role of MAPK3/1 in gap junction closure was not studied in these experiments. However, the effectiveness of both gonadotropins and epidermal growth factor (EGF) in gap junction deregulation [39] indicates that the EGF receptor (EGFR)/MAPK pathway is involved in this process in pigs.

Does cGMP Inhibit Meiotic Resumption through MAPK3/1 Signaling ?

Recent results indicate cyclic guanosine monophosphate (cGMP) plays a key role in the regulation of meiotic arrest and meiotic resumption in mammalian oocytes. Mural granulosa cells express a natriuretic peptide type C (NPPC), which serves as a ligand for natriuretic peptide receptor 2 (NPR2), a transmembrane guanylyl cyclase that is expressed in both mural granulosa and cumulus cells

[42, 43]. The synthesized cGMP is then transported through the gap junction from granulosa and cumulus cells into the oocyte and works as a natural inhibitor of phosphodiesterase 3A (PDE3A), maintaining a high intracellular level of cAMP and meiotic arrest of the oocyte [44–46]. The preovulatory surge of LH lowered cGMP production in somatic follicular cells and induced the closure of gap junctions between the cells. The resulting decrease in oocyte cGMP relieved the inhibition of PDE3A, and consequently, the oocyte cAMP level dropped from 700 to 140 nM, leading to the resumption of meiosis [45]. These data strongly indicate that cGMP plays a key role in the regulation of oocyte meiotic arrest and resumption. The drop in intercellular communication is necessary for meiosis re-initiation and is thought to limit the transfer of meiosis-blocking substances to the oocyte. In mice, the activation of EGFR is involved in the LH-induced decrease in cGMP transport from granulosa and cumulus cells to the oocyte, and both EGFR and MAPK3/1 activities participate in gap junction closure [37, 45, 46].

It has been proposed previously that cGMP may block meiotic resumption and cumulus expansion via a MAPK3/1-dependent mechanism. An activator of a soluble guanylyl cyclase and nitric oxide (NO) donor, S-nitroso-N-acetyl-penicillamine (SNAP), was found to prevent LH-induced MAPK3/1 activation in cultured rat follicles and to inhibit oocyte meiotic resumption and cumulus expansion, and correspondingly, a guanylyl cyclase inhibitor, 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one, mimicked the action of LH and induced MAPK3/1 phosphorylation [47]. In pig COCs, 8-bromo-cGMP (8-Br-cGMP) moderately inhibited FSH-induced oocyte maturation and cumulus expansion and partially reduced the phosphorylation of MAPK3/1 in both oocytes and cumulus cells [48]. However, the recent results of our laboratory demonstrate that neither endogenous cGMP nor exogenous cGMP analogues interfere with the FSH-induced activation of MAPK3/1, expression of cumulus expansion-related genes and degree of cumulus expansion in pigs [31]. A possible explanation for these contradictory results is that MAPK3/1 activation may be inhibited by NO itself, or its donors like SNAP, by a cGMP-independent mechanism. NO can block the activity of constitutively active RAS and RAF1 proteins [49] as well as the activity of EGFR [50].

The Role of MAPK 3/1 in the Regulation of Cumulus Cell Expansion

The participation of MAPK3/1 in cumulus expansion was first documented in the model of mouse *Mos*^{-/-} COCs, in which FSH-, EGF- and 8-Br-cAMP-induced expansion was blocked by U0126, indicating that the MAPK3/1-dependent step occurs downstream of the activation of PKA [21]. It is well known that the MAPK3/1 signaling cascade becomes activated by binding ligands to EGFR. We have shown previously that EGFR tyrosine kinase activity is essential for EGF-induced cumulus expansion of pig COCs, and that this activity can be modulated by pre-exposure of COCs with FSH [51]. These results strengthened the idea that EGF may have a physiological role in the regulation of cumulus cell expansion in preovulatory follicles, presumably as a mediator of LH signals [52]. This idea was modified by the finding that in preovulatory follicles, LH binds to receptors on mural granulosa cells and stimu-

lates the expression of EGF-like peptides [53, 54]. These peptides (amphiregulin-AREG, epiregulin-EREG and betacellulin-BTC) then act directly on cumulus cells, bind to the EGFR and stimulate the resumption of meiosis and expansion of cumulus cells in both follicle-enclosed and cumulus-enclosed oocytes. Thus, activation of the EGFR and MAPK3/1 appears critical for both gonadotropin- and EGF-like factor-induced cumulus expansion.

Inhibition of MAPK3/1 prevented a rise in the expression of expansion-related genes (*Has2*, *Ptgs2*, *Tnfrsf6*, *Ptx3*) required for normal cumulus expansion [22, 55, 56]. We have recently shown that inhibitors of EGFR (AG1478) and MAPK3/1 signaling (U0126) completely inhibited the AREG-induced expression of the expansion-related genes, but the FSH-stimulated expression of the genes was only partially decreased by these inhibitors [27]. Next, the activation of MAPK3/1 in preantral mouse granulosa cells is not sufficient to increase the expression of the expansion-related genes [55]. This indicates that additional signaling pathways are involved in the FSH-induced expansion of cumulus cells. Strong candidates are the MAPK14, phosphoinositide-3-kinase (PI3K) and prostaglandin E2 (PGE2)/PGE2 receptor pathways, all of which are activated in cumulus cells by FSH [27, 29, 57]. Specifically, the activation of PI3K by insulin growth factor 1 was shown to promote the FSH-stimulated synthesis and retention of hyaluronic acid in pig COCs [29]. Taken together, it is clear that the activation of MAPK3/1 is an essential but insufficient condition for the expansion of cumulus cells in rodents and pigs.

The Role of MAPK 3/1 in the Regulation of Steroidogenesis

The gonadotropin hormones dramatically change the pattern of steroid hormone production in preovulatory follicles and cultured cumulus-oocyte complexes. The steroid hormones produced by the granulosa and cumulus cells of large ovarian follicles, namely estradiol and progesterone, are indispensable for the development and function of reproductive organs, and the direct involvement of steroid hormones in the regulation of oocyte meiosis has been a matter of debate for decades. Recent evidence argues for an important role of the key steroid hormones in the regulation of both oocyte meiotic arrest and resumption. Estradiol promotes and maintains the expression of *Npr2* in the cumulus and periantral granulosa cells of mouse eCG-stimulated follicles, thereby preserving the ability of the cells to produce cGMP, which is required for the maintenance of meiotic arrest [58]. The progesterone signaling via its cognate receptor is essential for meiotic resumption and cumulus expansion in pigs [59]. The production of estradiol and progesterone in cumulus/granulosa cells is controlled by a gonadotropin-induced, cAMP- and PKA-dependent pathway [60]. However, there is increasing evidence that MAPK3/1 plays a dominant role in the expression of key steroidogenic enzymes responsible for the production of estradiol and progesterone. In mice stimulated with hCG, the expression of *Cyp19a1* is downregulated and the expression of *Star* and *Cyp11a1* is upregulated in both mural granulosa and cumulus cells, which corresponds with a decrease in circulating estradiol and increase in progesterone. The same pattern in the regulation of gene expression was observed in *in vitro* cultured COCs and granulosa cells stimulated

with gonadotropins or 8-Br-cAMP [61]. However, when the cultured cells were treated with U0126, the gonadotropin- or 8-Br-cAMP-induced production of progesterone and expression of *Star* and *Cyp11a1* were downregulated, whereas the estradiol production and expression of *Cyp19a1* were upregulated [61]. The same effect of U0126 on estradiol and progesterone secretion, accompanied by the upregulation of *Cyp19a1* and downregulation of *3Bhsd*, was observed in cultured pig COCs [62]. Thus these data indicate that in preovulatory follicles, an LH surge induces the differential expression of genes essential for estrogen and progesterone production in both cumulus and mural granulosa cells, and that this process is mediated by a MAPK3/1-dependent pathway.

A Lesson from Erk1/2 Double Mutant Mice

The most compelling evidence of the pivotal role of MAPK3/1 in the control of ovulation processes came from experiments with granulosa-cell-specific *Mapk3/1* double-knockout mice, the females of which fail to ovulate and are completely infertile [33]. Ovaries of *Erk1/2^{gc/-}* mice contained preovulatory follicles but no corpora lutea. Administration of hCG to these animals did not lead to cumulus expansion; their oocytes did not resume meiosis, and their follicles failed to ovulate and luteinize. The concentration of estradiol in serum was elevated, but that of progesterone was not. In concert with these observations, the expression of *Cyp19a1* was markedly increased, and the expression of two luteinization markers, *Star* and *Cyp11a1* was substantially reduced. Accordingly, the genes that are induced by FSH or eCG during preovulatory follicle development (*Fshr*, *Lhcgr* and *Nr5a2*) and normally downregulated by LH remained expressed at elevated levels in *Erk1/2^{gc/-}* mice. The expression of the genes associated with cumulus expansion and ovulation (*Has2*, *Tnfaip6*, *Ptgs2*, *Ptx3* and *Pgr*) was completely abolished in these *Mapk3/1* null mice. Thus MAPK3/1 is required for terminating the expression of the genes controlling the proliferation of granulosa cells as well as for inducing the genes controlling luteinization and ovulation.

Oocytes isolated from *Erk1/2^{gc/-}* mice spontaneously underwent GVBD in culture and reached metaphase II with the same frequency as the oocytes from control mice. However, when spontaneous GVBD was blocked by hypoxanthine, AREG stimulated GVBD and cumulus expansion in control COCs, but not in the COCs from *Erk1/2^{gc/-}* mice. These results clearly indicate that the oocytes of *Erk1/2^{gc/-}* mice retain MAPK3/1 and are capable of meiotic maturation but that the cumulus cells lacking MAPK3/1 fail to respond to EGF-like factors [33].

Signaling Pathways of Gonadotropin-induced Activation of MAPK3/1 in Cumulus and Granulosa Cells

The ability of gonadotropins and 8-Br-cAMP to activate MAPK3/1 in cultured granulosa cells was first demonstrated by Cameron *et al.* [63], indicating that an increase in intracellular cAMP is involved in this process. In mammalian preovulatory follicles, MAPK3/1 becomes activated after the LH surge through stimulation of the EGFR (ERBB1) by ligands produced by mural granulosa cells [53, 54]. The ligands, AREG, EREG and BTC, are EGF-family peptides,

and their expression at the mRNA level increased in cultured mouse follicles as early as 30 min after the addition of LH and reached its maximum after 2 h, in parallel with EGFR phosphorylation [64]. EGFR phosphorylation was inhibited by AG1478, an EGFR kinase inhibitor, and the matrix metalloprotease inhibitors GM6001 and Tapi1, indicating that ligand shedding is required for their activity. The activation of MAPK3/1 was inhibited by culturing the follicles in ligand-neutralizing antibodies and partially inhibited by AG1478 and GM6001, which suggest that other pathways are involved in this process in addition to the ligand-induced activation of the EGFR [64]. It is clear that the FSH-induced process of EGF-like ligand mRNA transcription, synthesis and shedding requires at least 0.5–1 h, but in a paper by Wayne *et al.* [65], gonadotropin-induced MAPK3/1 activation in cultured rat granulosa cells was reported to occur in 5–10 min. In addition, some LH-induced and MAPK3/1-dependent processes, such as gap junction closure in cultured mouse follicles [36], are known to occur within minutes. Therefore gonadotropins must be able to activate MAPK3/1 by an alternative pathway(s) that is quicker than the ligand-activated EGFR pathway. This alternative pathway(s) may either involve EGFR activation or skip EGFR and directly activate a signaling molecule upstream of MEK1. In cultured rat granulosa cells, the FSH-induced activation of Ras and MAPK3/1 required Src family tyrosine kinase (SFK) and EGFR tyrosine kinase activities, but not PKA or PI3K activity [65]. The involvement of SFKs in the activation of MAPK3/1 appears to be specific to gonadotropins, since the amphiregulin-induced activation of MAPK3/1 was not affected by SFK inhibition [65].

Another possible mechanism of the gonadotropin-induced rapid activation of MAPK3/1 may be direct activation of signaling molecules operating upstream of MEK1. Natural candidates for these molecules are the small GTP-binding proteins, such as Ras or Rap1, which interact with downstream molecules on the MAPK signaling cascade, c-Raf and B-Raf, respectively. The binding of gonadotropins to their specific G protein-coupled receptors catalyses the exchange of bound GDP to GTP in the α -subunit of the G-protein, which leads to release of the GTP-bound- α -subunit as well as the $\beta\gamma$ -subunit from the receptor. Both subunits can stimulate downstream signaling molecules, including the small GTPases, and thus regulate multiple signaling pathways including the MAPK3/1 pathway [66]. The role of G_s proteins in the regulation of adenylyl cyclase in granulosa cells is well documented [67], but very limited information exists about the role of G_i and G_q proteins in the regulation of ovarian functions. G_i proteins play a role in the ANP-mediated inhibition of the forskolin-induced maturation of pig oocytes [48], and G_q/11 signaling is required for LH-induced ovulation in mice [68]. G_i and G_q proteins can activate signal transduction kinases including PI3K and phospholipase C β and consequently protein kinase C (PKC). Data from several laboratories indicate that PKC may play a specific role in the gonadotropin-induced maturation of oocytes. PKC activators phorbol myristate acetate (PMA) and oleoyl-acetyl-sn-glycerol (OAG) inhibit spontaneous GVBD in denuded mouse oocytes but stimulate meiotic resumption in COCs [69]. A study of the mechanism of PKC action found that PKC activators activate MAPK3/1 in cumulus cells in the absence of FSH stimulation and that the MEK inhibitor U126 prevented GVBD in PMA- or OAG-stimulated COCs. Moreover, PKC inhibitors blocked FSH-induced

oocyte meiotic resumption and MAPK3/1 activation [70]. In pig COCs, PMA promoted oocyte GVBD, and the EGFR inhibitor AG1478 reversed this effect. Inhibition of PKC (by chelerythrine chloride) completely blocked FSH-induced meiotic resumption, but had no effect on EGF- or AREG-induced resumption [71]. In pigs, activation of TACE/ADAM-17 in a PKC-induced c-Src-dependent manner was shown to play a role in the proteolytic activation of EGF-like factors [72], but surprisingly, only after 20 h of culture [73]. Taken together, these data indicate that PKC participates in the FSH-induced transactivation of EGFR.

It has been shown recently that except the classical EGFR, ERBB1, other members of the EGFR family participate in transduction of LH signals in preovulatory follicles, inclusive of regulation of MAPK3/1 activity in granulosa and cumulus cells. Noma *et al.* [74] showed that ERBB2/ERBB3 complexes are phosphorylated in mouse preovulatory follicles 2 h after hCG administration, in parallel with increased expression of the ERBB3 ligand, neuregulin 1 (*Nrg1*), in mural granulosa cells. In vitro, the NRG1 protein induced activation of protein kinase B in cultured granulosa cells, but not activation of MAPK3/1. However, NRG1 synergistically enhanced AREG-induced phosphorylation of MAPK3/1, both in cultured granulosa cells and COCs. Co-treatment of cultured COCs with AREG and NRG1 delayed GVBD in oocytes without preventing oocyte progression to metaphase II and significantly enhanced the cleavage rate of fertilized oocytes as compared with the action of AREG alone [74]. The molecular background of the NRG1 action on oocyte maturation was elucidated in a model of granulosa and cumulus cell-specific *Nrg1* knockout mice [75]. These mice exhibited reduced fertility, accelerated maturation of oocytes and inability to arrest meiosis at the metaphase II stage, which was associated with a high level of intracellular calcium, increased activity of PKC and increased phosphorylation of connexin 43 in granulosa and cumulus cells and reduced levels of phosphorylated MAPK3/1 in ageing oocytes. Taken together, these studies revealed that early induction of *Nrg1* in preovulatory follicles is required for precise regulation of PKC and MAPK3/1 activities and is essential for normal progression of meiosis and acquisition of cytostatic activity and thereby for successful fertilization and embryo development [74, 75].

Gonadotropin-induced signaling pathways leading to the activation of MAPK3/1 in mammalian follicles are summarized in Fig. 1.

Conclusions and Perspectives

To date, G_s -, cAMP-, PKA- and ADAM-17-dependent production of EGF-like factors and consequent activation of the EGFR/MAPK3/1 pathway have been clearly elucidated in mammalian preovulatory follicles, but an alternative PKA-independent pathway involving Src kinase-mediated activation of the EGFR has also been described. To characterize the gonadotropin-induced signals leading to the activation of MAPK3/1 in the granulosa/cumulus cells of preovulatory follicles, the association of different classes of G-proteins (G_s , G_i and G_q) with the gonadotropin receptors should be elucidated. Next, the involvement of signal transduction kinases, either cAMP dependent (PKA) or cAMP independent (via Src, PI3K/Akt and PLC β /PKC), in the regulation of small GTP-binding proteins (Rap1, Ras) and MEK-activating kinases (B-Raf, c-Raf) should be investigated

to determine the complex signaling network participating in the activation of MAPK3/1 in follicular somatic cells.

Once activated, MAPK3/1 plays a critical role in the key events of final follicle maturation. It regulates transport of meiosis-arresting substances in follicular somatic cells and between the somatic cells and oocyte through modulation of gap junction permeability. Next, it affects the production of steroid hormones, the synthesis of cumulus cell extracellular matrix proteins and the activity of various regulatory molecules through the modulation of granulosa and cumulus cell transcriptional activity. Thus, the activity of MAPK3/1 becomes essential for meiosis resumption, cumulus expansion and ovulation of the matured oocyte. The role of MAPK3/1 in each of these processes is critical, but cooperation with other cellular protein kinases is required. The role of specific protein kinases in the regulation of various transcription factors should be thoroughly investigated to determine the complexity of gonadotropin-induced signaling in preovulatory follicles.

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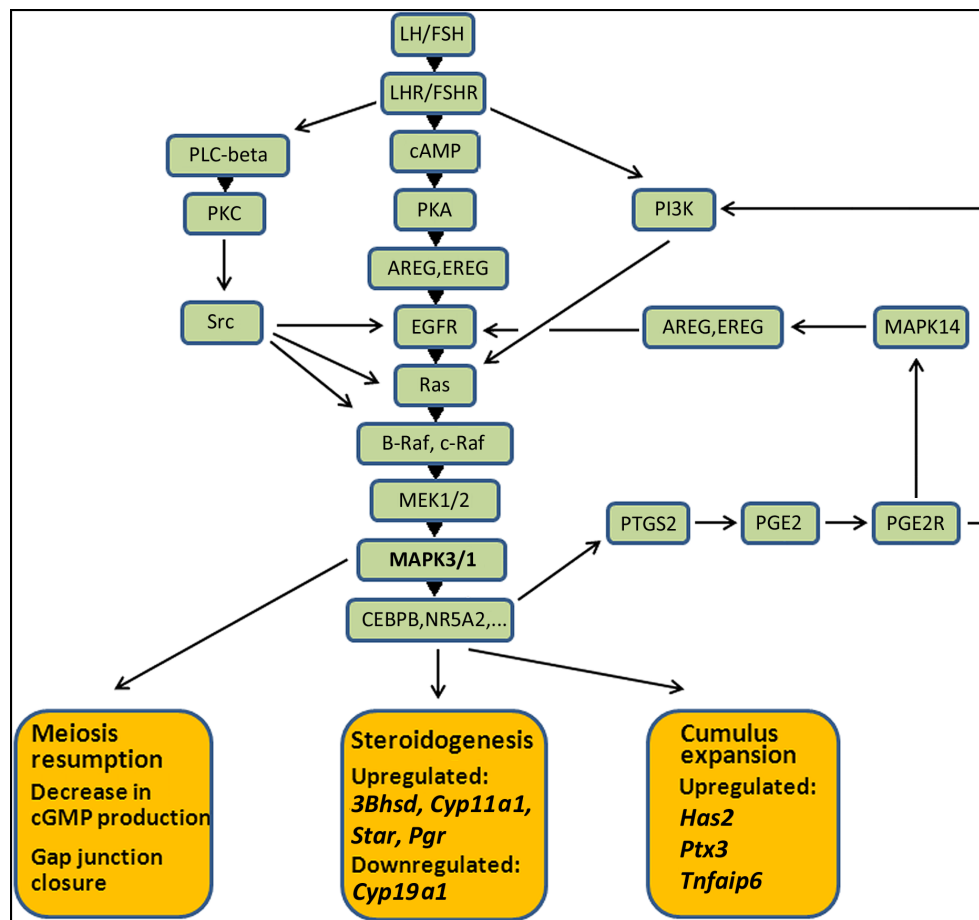


Fig. 1. Signaling pathways leading to gonadotropin-induced activation of MAPK3/1 in preovulatory follicles or cultured cumulus-oocyte complexes. The pathways are activated by LH, *in vivo* or *in vitro*, in the model of whole-follicle cultures. In the follicles, LH-induced generation of EGF-like peptides (AREG, EREG) occurs in mural granulosa cells, and EGFR-related signaling may occur in both mural granulosa and cumulus cells. The depicted pathways were also identified in cultured cumulus-oocyte complexes stimulated by FSH. The gonadotropins bind to their receptors and activate G-proteins that ensure transduction of the signals to effector enzymes and kinases. The activation of MEK/MAPK upstream kinase (B-Raf, c-Raf) occurs via ligand- (AREG) or PKC/Src-induced transactivation of EGFR. Whether Src- or PI3K-mediated activation of Ras or Raf, passing by EGFR, exist in mammalian follicles, has yet to be confirmed. The prolonged activity of EGFR/MAPK3/1 observed in gonadotropin-stimulated granulosa and cumulus cells is maintained by regulatory feedback loops involving the production of PGE2 and signaling via PGE2R. This signaling involves a MAPK14 inhibitor-sensitive expression of EGF-like peptides, which bind to EGFR, and the activation of PI3K/AKT, the target of which needs to be specified. The resumption of oocyte meiosis is triggered by posttranscriptional mechanisms, comprising mainly phosphorylation/dephosphorylation events affecting the production and transport of cGMP in the cumulus-oocyte complex. However, a global change in the granulosa/cumulus cell transcriptomes is essential for successful completion of the maturation and ovulation processes. The transcription factors identified so far are depicted, as well as some of the genes participating in the regulation of granulosa/cumulus cell functions associated with oocyte maturation and cumulus expansion. The figure is predominantly based on studies carried out on rodent and pig models; some parts of the depicted pathway have been confirmed in other mammalian species.

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