Review

Signaling Pathways in Insulin- and IGF-I Mediated Oocyte Maturation in Lower Vertebrates

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Received 06 July 2014; revised 26 November 2014

The endocrine control of oocyte maturation in fish and amphibians has proved to be a valuable model for investigating the rapid and non-genomic steroid actions at the cell surface. Considerable progress has been made over the last decade in elucidating signaling pathways in steroid-induced oocyte maturation. In addition to steroids, various growth factors have also been reported to be involved in this process and progress being made to elucidate their mechanism of actions. Exposure of fully-grown oocytes to steroids or growth factors (insulin/IGFs) initiates various signaling cascade, leading to formation and activation of maturation-promoting factor (MPF), a key enzyme that catalyzes entry into M-phase of meiosis I and II. Whereas the function of MPF in promoting oocyte maturation is ubiquitous, there are differences in signaling pathways between steroids- and growth factors-induced oocyte maturation in amphibian and fish. Here, we have reviewed the recent advances on the signaling pathways in insulin- and IGF-I-induced oocyte maturation in these two groups of non-mammalian vertebrates. New findings demonstrating the involvement of PI3 kinase and MAP kinase in induction of oocyte maturation by insulin and IGF-I are presented.

Keywords: Amphibia, Fish, Insulin, IGF-I, Oocyte maturation, Signaling pathways

Introduction

Fully grown oocytes get arrested at prophase of first meiosis and in echinoderms and lower vertebrates species-specific maturation inducing steroid (MIS) relieves oocytes from this arrest. Oocytes then undergo a process of meiotic maturation, termed oocyte maturation (OM) to produce an egg that can be fertilized. Progesterone is considered to be the most potent MIS in amphibians, whereas it is 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) in most fishes (for review see). However, in sciaenid and some other fishes 17,20β, 21-trihydoxy-4-pregnen-3-one (20β-S) has been identified as MIS. Steroid-triggered OM in lower vertebrates occurs independent of transcription through a G protein-coupled membrane receptor which triggers rapid changes in the intracellular signaling cascades that ultimately converge to the activation of a dimeric protein kinase, the maturation promoting factor (MPF), a complex formed by cyclin B and cdc2 kinase. Active MPF promotes progression from meiotic MI-MII through H1 kinase activation. Although the function of MPF in promoting OM is ubiquitous, there are species-specific differences in the signaling events that lead to MPF activation.

In addition to MIS, insulin and insulin-like growth factors (IGFs) have been shown to act as potent regulators of meiotic maturation in fish and amphibian. Importantly, while significant progress has been made in identifying the steroids, steroid receptors and intracellular signaling pathways that regulate OM, the signaling cascades in insulin- and growth factor-induced OM in lower vertebrates are not fully explored.

Here, we provide a brief overview of meiotic progression in fish and amphibian oocytes in response to insulin and IGF-I and present novel data, implicating classical growth factor receptors as important regulators of insulin- and IGFs-triggered...

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Abbreviations: GVBD, germinal vesicle breakdown; IGF-I, insulin-like growth factor; IGFs, insulin-like growth factors; IRS-I, insulin and IGF-I receptor substrate; MAP kinase, mitogen-activated protein kinase; MPF, maturation promoting factor; OM, oocyte maturation; PI3 kinase, phosphatidylinositol kinase; PIP3, phosphatidylinositol 3, 4, 5-triphosphate; PH, pleckstrin homology; 17,20β-P, 17,20β-dihydroxy-4-pregnen-3-one.
OM in fish. Firstly, we focus on steroid-induced OM before considering the signaling pathways of this event after insulin/IGFs-stimulation in amphibians and fish oocytes.

Steroid-induced oocyte maturation

In fish and amphibians, OM is initiated by binding of MIS to a novel G protein-coupled membrane progesterin receptor (mPRα) on the oocyte surface and activates various signal transduction pathways, presumably through inhibition of cAMP-dependent protein kinase (Prka) activity, ultimately leading to GVBD. Another signaling molecule activated during OM in many species is phosphatidylinositol 3 kinase (PI3 kinase). Activation PI3 kinase is necessary for MIS-induced OM in starfish, amphibians and fish.

Fish and amphibian MPF

Fish MPF like that of Xenopus consists of two components: one is a homolog of cdc2+ gene product of fission yeast (Sacharomyces pombe) referred to as p34cdc2 (cdc2) and other is cyclin B+. Fully grown oocytes of amphibian contain a stockpile of pre-formed cyclin B-cdc2 kinase, which is maintained in an inactive form (pre-MPF) by inhibitory phosphorylations of Thr14 and Try15 of cdc2 (see review). In contrary, goldfish oocytes contain an inactive monomeric 35 kDa cdc2 without cyclin B and during maturation they contain both an active 34 kDa and an inactive 35 kDa cdc2 kinases. MIS induces de novo synthesis of cyclin B that binds to 35 kDa cdc2 and allows MO15 to phosphorylate cdc2 on Thr161 by inducing an electrophoretic mobility shift from 35 to 34 kDa. Thereafter, Ser79 and/or Ser94 of cyclin B is phosphorylated by active cyd2, yielding MPF (see review). These mechanisms of MPF activation in fish apparently differs from those of Xenopus.

Role of MAP kinase

Activation of MAP kinase is universal during OM, although its requirement for GVBD is uncertain. MAP kinase plays an important role in the process of steroid-induced OM in Xenopus. Involvement of MAP kinase in 2-hydroxyestradiol-17β-induced OM in catfish Heteropneustes fossilis has been reported. Khan and Moitra have shown the participation and activation of cAMP-dependent protein kinase and MAP kinase in MIS-induced OM in Anabas testudineus. Although several earlier studies have demonstrated the participation of Mos/MAP kinase in metaphase II arrest, involvement of MAP kinase in progesterone-induced MPF activation and GVBD-induction is species-dependent in fish, amphibian and mammalian model.

Insulin- and IGF-I-induced oocyte maturation

Insulin, IGF-I and their receptors

Insulin, IGF-I and other growth factors in fish and amphibians regulate a wide range of cellular function through activation of their membrane receptors with tyrosine kinase activity. Ovarian regulation is supported by the presence of their membrane receptor tyrosine kinase (IGF-I receptor) by the ability to produce IGF-I and by the presence of IGF-I in the circulation. With regards to the possible source of insulin, it is not certain whether fish and amphibian ovary can produce insulin, but the major source of insulin is most likely the pancreas and perhaps also the adipose tissue. Therefore, an interesting link between IGF-I and insulin-regulated metabolic and growth processes, ovarian steroid production and OM can be suggested as proposed earlier.

PI3 kinase activation is required for insulin- and IGF-I-mediated OM in amphibian

Insulin and IGF-I induce amphibian OM through binding and activation of their membrane receptor tyrosine kinase and triggers various downstream signaling events. Several of the proteins and enzymes in linking the insulin receptors to its final effects in Xenopus oocyte have been elucidated. Two of the earliest components in this pathway are insulin and IGF-I receptor substrate-I (IRS-I) and the enzyme PI3 kinase. Following insulin or IGF-I stimulation, IRS-I rapidly associates with and activate PI3 kinase by interaction between highly conserved phosphopeptide sequence motifs of IRS-I and SH2 domain of 85 kDa subunit of PI3 kinase.

PI3 kinase catalyzes the production of phosphatidylinositol 3,4,5-triphosphate (PIP3) from plasma membrane lipid phosphatidylinositol 4,5-bisphosphate. The family of PI3 kinases comprise of 14 enzymes separated into four classes, of which classes I (A and B), II and III are lipid kinases and class IV are protein kinase. Class I PI3 kinase is composed of a regulatory 85 subunit and a catalytic p110α or p110β subunit. The regulatory
85 kDa subunit of class IA PI3 kinase stabilizes and inactivates the p110 catalytic subunit under resting conditions \(^7^4\) and upon activation, p85 relieves the inhibitory activity on p110 \(^7^5\).

Earlier, it has been shown that class I PI3 kinase is likely to mediate growth factor-induced OM in *Xenopus* \(^7^6\). In starfish, *Xenopus* and mouse OM, activation of PI3 kinase and formation of PI(3,5)P\(_3\) recruits some intracellular signaling proteins that contain pleckstrin homology (PH) domain, such as serine/threonine kinase Akt to plasma membrane \(^6^9\), and this is sufficient to induce OM in these species \(^2^3,7^7,7^8\). It has been shown that one specific downstream target of PI3 kinase/Akt is the activation of oocyte-specific phosphodiesterase that degrades cAMP and activation of oocyte specific phosphodiesterase, which is found to mediate IGF-I, not steroid-induced oocyte maturation in *Xenopus* \(^3^3,2^4\). Thus, it appears from available literature that PI3 kinase activation is essential for insulin/IGFs-induced OM in amphibians.

**PI3 kinase activation is required for insulin- and IGF-I-induced OM in fish**

Previous studies using full grown oocytes of fish have demonstrated that both insulin and IGF-I at their increasing concentrations cause significant stimulation of OM \(^2^0-2^2,3^1,7^9-8^1\). Potential mechanisms underlying insulin/IGFs-induced OM in fish include their effects on ovarian MIS production, which in turn induces OM and development of oocyte maturational competence \(^6^2-8^4\). In red seabream, mummichog, striped bass and common carp, insulin and IGF-I could induce OM in fully denuded oocytes or in intact follicles in presence of steriodogenesis inhibitors \(^2^0,2^2,8^0,8^5\), indicating the physiological relevance of insulin/IGF on final OM through a pathway independent of steroid action.

First evidence for the involvement of PI3 kinase in insulin- and IGF-I-induced OM in fish is available from the study using two mechanistically different PI3 kinase inhibitors, either wortmannin or LY294002. These two inhibitors at their increasing concentrations sufficiently inhibited insulin/insulin-I-induced OM in fully denuded oocytes in fish \(^2^0,2^2,3^1\) (Fig. 1).

Unlike *Xenopus*, evidence for participation of membrane tyrosine kinase receptor in insulin/IGFs-induced OM in fish is very limited. Our recent result with common carp oocytes provides a preliminary evidence for activation membrane receptor with tyrosine kinase in insulin- and IGF-I-induced OM which is presented here. We used fully-denuded oocytes obtained from full-grown ovarian follicles of carp after a pre-incubation with 17\(\beta\)-estradiol (E2) for 2 h as described previously \(^3^2\) and incubated with increasing concentration of receptor tyrosine kinase inhibitor (5, 10, 15 and 20 \(\mu\)M) in presence or absence of either IGF-I (25 nM) or insulin (0.8 IU/ml) for 8 h and examined the GVBD. Further, we used E2 because enzymatic or manual removal of follicle layers in fish oocytes caused marked spontaneous OM, which could be partially reversed by the treatment of E2 \(^8^6,3^2\).

Microscopic examination of cleared oocytes (clearing solution—6:3:1, methanol:formalin:acetic acid) has shown gradual and significant \((p < 0.05)\) attenuation of insulin- and
IGF-I-induced OM in presence of graded doses of inhibitor (Fig. 2). Results suggest that insulin and IGF-I mediate their action by binding to receptor tyrosine kinase and PI3 kinase inhibitor inhibit OM by directly inhibiting PI3 kinase within IGF-I and insulin receptor signal transduction pathway.

**PI3 kinase activation and effects of PI3 kinase inhibitors**

In fish oocytes studied so far, full activation of PI3 kinase by insulin and IGF-I takes place within 90 min of their treatments both in intact follicles and denuded oocytes, followed by GVBD induction. The findings that incubation of oocytes with PI3 kinase inhibitors, wortmannin and LY294002 sufficiently blocks PI3 kinase activation within 90 min, followed by inhibition of GVBD support the requirement and activation of PI3 kinase in IGF-I- and insulin-induced OM in fish. At present, it is not known whether insulin and IGF-I in fish oocytes, like that of *Xenopus* activate PI3 kinase via IRS-I protein that associates PI3 kinase through interaction between YMXM/YYXM sequence motif and SH2 domain. Further studies, therefore, are required to confirm the involvement of IRS-I in ligand-stimulated PI3 kinase in fish OM.

It has been demonstrated that in carp oocyte IGF-I- and insulin-induced PI3 kinase activation leading to GVBD induction takes place several hours before cdc2 is activated. Immunoblot studies using cdc2 p34 antibody has revealed that IGF-I and insulin induces cdc2 activation by converting inactive cdc2 p35 to high activity stage cdc2 p34 and maximum activation is recorded at 21 h after incubation. Both the inhibitors of PI3 kinase inhibit cdc2 kinase activation induced by insulin and IGF-I, leading to OM after 21 h incubation. Moreover, a very recent study has also shown that insulin-induced OM in zebrafish oocytes involves activation of PKB/Akt, an event which is sensitive to PI3 kinase activation. Using phosphospecific antibodies a large and rapid activation of the zebrafish Akt upon insulin stimulation has been observed. Furthermore, time kinetics of PI3 kinase (30-60 min) and Akt (45-120 min) activation show tight temporal relationship and is comparable to common carp and mouse oocyte. Taken together, these results suggest that Akt is a downstream regulator of PI3 kinase which may have decisive role in allowing insulin-induced G2-M transition in fish oocytes.

Involvement of a cAMP-independent signaling cascade has been proposed in starfish, Atlantic croaker and *Rana dybowskii*, as PI3 kinase inhibition could successfully block MIS-induced maturation. Our study and a recent report have also proposed the involvement of cAMP-independent signaling cascade for insulin- and IGF-I-induced OM in carp and zebrafish. It may therefore reasonable to think that there might be separate signaling pathways available for cdc2 activation in response to different mitogens in fish.

**Requirement of MAP kinase in insulin- and IGF-I-induced OM**

Mitogen-activated protein kinase (MAP kinase) is rapidly activated in response to hormones and growth factors for diverse biological function. Previously, it has been shown that activation of MAP kinase is required for progesterone-induced OM in *Xenopus*. Later studies with *Xenopus* and mouse oocytes using various MAP kinase inhibitors have shown...
that although MAP kinase is activated during OM its requirement in GVBD is uncertain. Available information also indicates that MAP kinase activation is not necessary for MIS-induced OM in goldfish and Atlantic croaker. In contrast, some recent studies have reported the activation of MAP kinase in MIS-induced OM in *Heteropneustes fossilis* and *Anabas testudineus*. Although several studies have demonstrated Mos/MAP kinase in metaphase-II arrest, involvement of MAP kinase in MIS-induced MPF activation is species-dependent in fish, amphibian and mammalian model.

Studies on the involvement of MAP kinase in insulin- and IGF-I-stimulated cdc2 activation and OM are very limited. Earlier studies have demonstrated the activation of MAP kinase in insulin- and IGF-stimulated OM in *Xenopus*. Our recent study has also demonstrated that MAP kinase is rapidly and strongly activated after IGF-I and insulin stimulation in common carp and this activation is specific to the induction of GVBD which takes place several hours before cdc2 becomes activated.

Western blot analysis of oocyte lysate using phosphospecific ERK1/2 antibody shows that ERK1/2 phosphorylation in these oocytes is increased by the stimulation of IGF-I and insulin in a time-dependent manner and maximum phosphorylation occurs between 150-180 min. Furthermore, phosphorylation of ERK1/2 by IGF-I and insulin is mediated by the activation of upstream MAP kinase kinase (MEK), as has been shown by decreased ERK1/2 phosphorylation in presence of a specific MEK inhibitor PD98059.

Interestingly, MEK inhibitor PD98059 by blocking MAP kinase activation inhibits IGF-I- and insulin-induced cdc2 activation and OM in carp oocytes. Thus, in carp oocytes during insulin- and IGF-I-induced OM, the rise in cdc2 activity is coincident with phosphorylation and activation of MAP kinase. Therefore, evidences obtained so far in amphibian and fish indicate that activation of MAP kinase may be an important component for IGF-I and insulin-induced signal transduction cascade, leading to activation of cdc2 kinase and induction of GVBD. On the basis of available findings, a model (Fig. 3A and B) for generalized signaling pathways for PI3 kinase activation of oocytes through insulin/IGF-I receptor is proposed.

Conclusion

This brief review highlights the current picture of the mechanism of insulin and IGF-I regulation of OM in fish and amphibians. Both insulin and IGF-I can induce OM independent of steroid by directly acting on oocyte membrane. Specific receptors for insulin/IGFs have been documented on oocyte membrane. In animal kingdom, many connections between ligand-receptor complex and cdc2 kinase cascades are established during OM. Insulin- and IGF-mediated OM in fish and amphibian seem to be controlled by IRS-I and PI3 kinase. Evidence obtained in carp oocytes indicates that activation of MAP kinase is important for IGF-I and insulin-induced signal transduction cascade, leading...
to activation of cdc2 kinase and GVBD induction. As insulin- and IGF-I-induced MAP kinase activation and OM in carp is inhibited by PI3 kinase inhibitor, it appears that PI3 kinase possibly regulate MAP kinase signaling cascade. The requirement of MAP kinase activation in insulin- and IGF-I-induced OM in *Xenopus* is still not certain and further study is required to resolve this issue.

Acknowledgement

This research is supported by University of Kalyani, Kalyani. The authors acknowledge DST-PARS, University of Kalyani for providing partial financial support.

References
