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Authors: Satake, Honoo, Kawada, Tsuyoshi, Osugi, Tomohiro, Sakai, Tsubasa, Shiraishi, Akira, et al.

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[REVIEW]

Ovarian Follicle Development in Ascidians

Honoo Satake*, Tsuyoshi Kawada, Tomohiro Osugi, Tsubasa Sakai,
Akira Shiraishi, Tatsuya Yamamoto, and Shin Matsubara

Bioorganic Research Institute, Suntory Foundation for Life Sciences, Kyoto, Japan

Ovarian follicle development is an essential process for continuation of sexually reproductive animals, and is controlled by a wide variety of regulatory factors such as neuropeptides and peptide hormones in the endocrine, neuroendocrine, and nervous systems. Moreover, while some molecular mechanisms underlying follicle development are conserved, others vary among species. Consequently, follicle development processes are closely related to the evolution and diversity of species. *Ciona intestinalis* type A (*Ciona rubusta*) is a cosmopolitan species of ascidians, which are the closest relative of vertebrates. However, unlike vertebrates, ascidians are not endowed with the hypothalamus-pituitary-gonadal axis involving pituitary gonadotropins and sexual steroids. Combined with the phylogenetic position of ascidians as the closest relative of vertebrates, such morphological and endocrine features suggest that ascidians possess both common and species-specific regulatory mechanisms in follicle development. To date, several neuropeptides have been shown to participate in the growth of vitellogenic follicles, oocyte maturation of postvitellogenic follicles, and ovulation of fully mature follicles in a developmental stage-specific fashion. Furthermore, recent studies have shed light on the evolutionary processes of follicle development throughout chordates. In this review, we provide an overview of the neuropeptidergic molecular mechanism in the premature follicle growth, oocyte maturation, and ovulation in *Ciona*, and comparative views of the follicle development processes of mammals and teleosts.

Key words: ascidian, *Ciona intestinalis* Type A, follicle, maturation, peptide, ovulation

INTRODUCTION

Ascidians are marine invertebrates that belong to the phylum Urochordata and superphylum Chordata, and are recognized as one of the closest relatives of vertebrates (Delsuc et al., 2006; Denoeud et al., 2010; Satoh et al., 2014). The cosmopolitan species *Ciona intestinalis* Type A (synonym for *Ciona rubusta*) has been employed as a model organism in various fields, including developmental biology, which have been markedly enhanced by genome sequencing (Dehal et al., 2002; Satou et al., 2005), analysis of gene expression profiles (Imai et al., 2004, 2006, 2012; Azumi et al., 2007; Kawada et al., 2017; Matsubara et al., 2021; Kawada et al., 2022), characterization of the developmental and/or functional transcriptional network in embryos and larvae (Satoh, 2003; Lemaire, 2011; 2008, Horie et al., 2018; Cao et al., 2019; Satou et al., 2019; Liu and Satou, 2020), and the development of transgenic and gene-edited *Ciona* (Sasakura et al., 2003, 2017; Sasakura and Horie, 2023). These studies have contributed a great deal to the investigation of embryonic development and metamorphosis of *Ciona* and the evolutionary history of chordates. In contrast, our knowledge of follicle development pathways in *Ciona* is lim-

ited.

Ciona follicles are classified into four developmental stages: pre-vitellogenic (stage I), vitellogenic (stage II), post-vitellogenic (stage III), and mature egg (stage IV) stages (Burighel and Cloney, 1997; Prodon et al., 2006; Matsubara et al., 2020). Stage-I follicles (Fig. 1) harbor a single oocyte with a diameter of less than 100 μm , and contain no yolk protein. Mitochondria are observed around the germinal vesicle (GV). Stage-II follicles (Fig. 1) harbor an oocyte with a diameter of around 110 μm , and begin to accumulate yolk proteins. Furthermore, multiple types of accessory cells, such as test cells and follicle cells, are developed and surround an oocyte (Fig. 1). Follicle cells have a cuboid shape, and mitochondria are distributed throughout the oocyte (Fig. 1). At stage III, follicles have grown with approximately 130- μm oocyte diameter with cone-shaped follicle shape, and become rich in yolk proteins, leading to the localization of mitochondria to the inner margin of the plasma membrane (Fig. 1). Fully grown stage-III follicles undergo GV breakdown (GVBD) and ovulation, and develop to fertile egg (Stage IV, approximately 150- μm oocyte diameter) (Fig. 1). However, no regulatory factors for *Ciona* follicle development were identified until we started to study them.

In early studies of ascidian neuroendocrine and nervous systems, cognate receptors for several *Ciona* neuropeptides, such as tachykinin (TK), gonadotropin-releasing hor-

* Corresponding author. E-mail: satake@sunbor.or.jp
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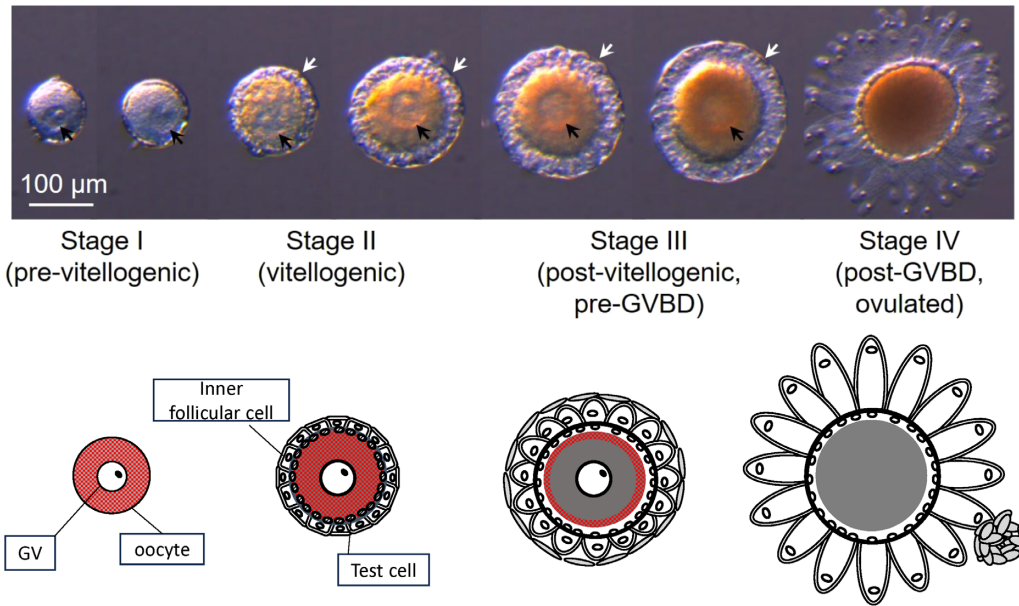


Fig. 1. Developmental stages of *Ciona* ovarian follicles. The ovary of adult ascidians contains numerous follicles at all developmental stages. Black and white arrows indicate germinal vesicle (GV) and outer follicular cell layers, respectively. Red dots indicate mitochondria. Permission has been obtained from the publisher to partially reproduce the image of follicles here (Matsubara et al., 2020).

mones (GnRHs), and vasopressin (VP) were found to be expressed in the ovary (Satake et al., 2004; Tello et al., 2005; Kawada et al., 2008), suggesting that these neuropeptides participate in some reproductive functions. Furthermore, neuroanatomical analyses using protein convertase 2 (a canonical peptide precursor processing enzyme) promoter-Kaede-transgenic *Ciona* adults revealed that peptidergic neurons in the cerebral ganglion innervate the ovary via the dorsal nerve cords (Osugi et al., 2017, 2020). Unlike vertebrate chordates, ascidians do not have the hypothalamic-pituitary-gonadal (HPG) axis or pituitary hormones gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) that play central roles in the regulation of maturation and ovulation in vertebrates, as discussed in the following sections. These findings support the view that ascidian follicle development is regulated by a variety of neuropeptides secreted from the cerebral ganglion in a neuroendocrine manner. Over the past 15 years, the molecular mechanisms underlying follicle development in *Ciona* has been explored. In this review, our goal is to provide a comprehensive summary and update on our current understanding of *Ciona* follicle growth, maturation, and ovulation, and to consider the evolutionary implications of these processes.

Premature follicle growth in *Ciona*

The vertebrate follicle development process is categorized into two pathways: the gonadotropin-independent pathway and gonadotropin-dependent pathway. In mammals, the former involves the growth of primordial, primary, and secondary follicles, and functions prior to puberty, including during the fetus, baby, and infancy stages (Robker et al., 2018; Duffy et al., 2019; Orisaka et al., 2021). The latter includes the growth of preantral, and antral follicles, maturation (GVBD) of Graafian follicles, and ovulation, and is trig-

gered by the release of gonadotropins from the onset of puberty to menopause, as stated below (Robker et al., 2018; Duffy et al., 2019; Orisaka et al., 2021). In oviparous animals such as teleost fish, follicle development is classified based on vitellogenesis. For example, in medaka, the previtellogenic and early vitellogenic stages are gonadotropin-independent, and the later vitellogenic and postvitellogenic stages are gonadotropin-dependent (Takahashi et al., 2019; Takahashi and Ogiwara, 2023). In mammals, phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), phosphatase and tensin homolog (PTEN), and anti-Müllerian hormone (AMH) are thought to be involved in activation of primordial follicles (Robker et al., 2018; Duffy et al., 2019; Orisaka et al., 2021). Moreover, the Transforming growth factor β (TGF β) superfamily proteins, such as Growth/differentiation factor 9 (GDF9) and Bone morphogenetic protein 15 (BMP15), and insulin-like growth factor 1 (IGF1) are considered to be involved in gonadotropin-independent follicle growth as well as gonadotropin-dependent growth (Robker et al., 2018; Duffy et al., 2019; Orisaka et al., 2021). However, no regulatory factor specific to gonadotropin-independent follicle growth has so far been identified.

In *Ciona*, the follicle development at stage I and stage II is expected, at least in part, to correspond to the gonadotropin-independent follicle growth in vertebrates. In the *Ciona* ovary, the only *Ciona* tachykinin (CiTK) cognate receptor, CiTKR, was observed exclusively in test cells of stage II follicles (Aoyama et al., 2008; Matsubara et al., 2016). Furthermore, as depicted in Fig. 2, CiTK was shown to up-regulate the gene expression and enzymatic activities of cathepsin D, chymotrypsin, and carboxypeptidase B1 (Aoyama et al., 2008; Matsubara et al., 2016), and to eventually induce the follicle growth from stage II to stage III (Aoyama et al., 2008; Matsubara et al., 2016). Of particular interest is that these three proteases are involved in proteolytic processing of

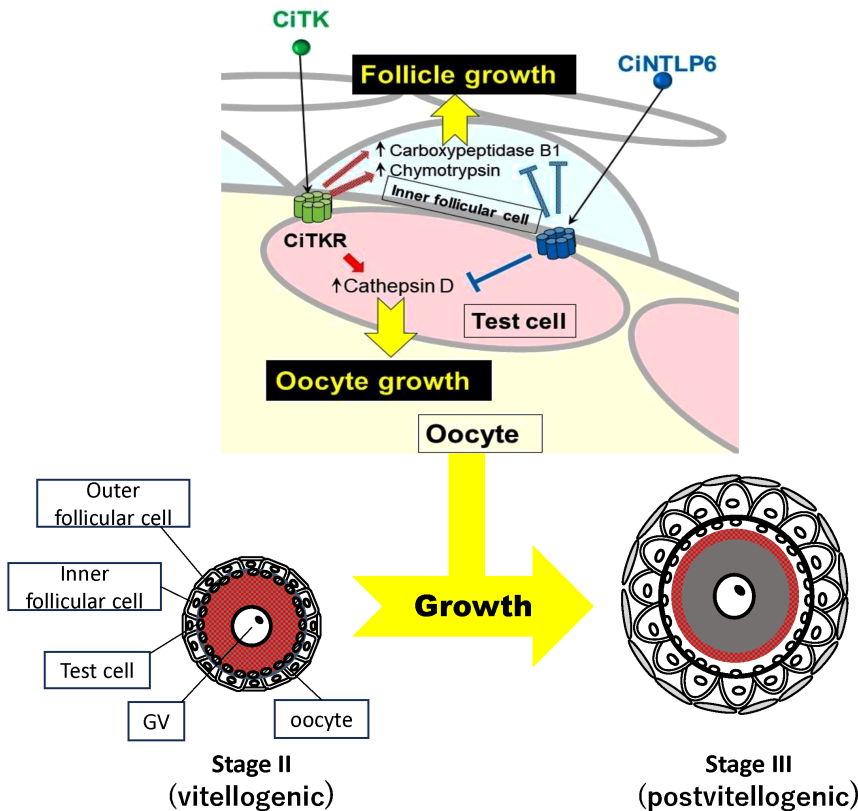


Fig. 2. Follicle growth from stage II to stage III. CiTK directly induces the gene expression of Cathepsin D in test cells. Moreover, CiTK secondarily upregulates the gene expression of Chymotrypsin and Carboxypeptidase B1 in inner follicular cells. Activation of these proteases is required for progression from stage II to stage III. Red dots indicate mitochondria.

oocyte components. Carboxypeptidase B1 is responsible for the proteolytic processing of several component proteins of zona pellucida in mammalian oocytes at an early growth stage (Litscher et al., 1999). Inhibition of chymotrypsin has been shown to suppress oocyte growth at preGVBD stages in a non-*Ciona* ascidian (*Halocynthia roretzi*) (Sakairi and Shirai, 1991), a starfish (*Asterina pectinifera*) (Takagi-Sawada et al., 1989; Tanaka et al., 2000), and the fruit fly (*Drosophila melanogaster*) (Jakobsen et al., 2005). Moreover, cathepsin D has been shown to proteolytically modify precursors of yolk proteins (e.g., vitellogenin) and follicular components in non-mammalian vertebrate oocytes at the preGVBD stages (Carnevali et al., 2006). These findings support the view that the three CiTK-induced proteases also participate in the enhancement of the growth of *Ciona* stage-II (namely, preGVBD stage) follicles via proteolysis of various oocyte and follicle proteins, including vitellogenin. Interestingly, cathepsin D is specifically co-localized with CiTKR in test cells of stage-II follicles, whereas gene expression of carboxypeptidase B1 and chymotrypsin was detected in the follicular cells of the stage-II and stage-III follicles (Aoyama et al., 2012). Furthermore, the cathepsin D gene expression occurred 3 h before the expression of carboxypeptidase B1 and chymotrypsin (Aoyama et al., 2012). These findings suggest that the cathepsin D gene expression is directly upregulated by CiTK in test cells at stage II, and that the two remaining protease genes are secondarily activated. It is

also noteworthy that the CiTK-I-directed stage II follicle growth was shown to be suppressed by a *Ciona*-specific neurotensin-like peptide, CiNTP6 (Fig. 2), via downregulation of the gene expression of carboxypeptidase B1, chymotrypsin, and cathepsin D (Kawada et al., 2011; Matsubara et al., 2016; Kawada et al., 2022; Satake, 2023). Collectively, these studies have substantiated the molecular mechanism underlying tachykinergic premature (preGVBD) follicle growth in *Ciona* (Fig. 2), although investigation of the molecular mechanism by which signaling by CiTK and CiNTP6 in test cells regulates the expression of carboxypeptidase B1 and chymotrypsin in inner follicular cells awaits further studies. To date, no experimental evidence for a biological role of TKs in follicle development in vertebrates has been provided. However, combined with the findings that the TK receptor genes are expressed in the ovary (Pintado et al., 2003; García-Ortega et al., 2014; Blasco et al., 2020, 2023), it is likely that tachykinergic early-stage follicle growth is conserved in vertebrates.

Oocyte maturation in *Ciona*

Oocyte maturation, a process involving drastic nuclear and cytoplasmic reorganization including resumption of meiosis I, is featured by GVBD upon prophase arrest of meiosis. In mammals, LH and FSH, secreted by the pituitary in response to a hypothalamic hormone, GnRH, stimulate steroidogenesis in the preantral follicles, leading to preantral-to-antral transition; FSH stimulates the synthesis of testosterone in theca cells, and the latter induce 17 β -estradiol (estrogen, E2) in granulosa cells using testosterone synthesized by theca cells (Robker et al., 2018; Duffy et al., 2019; Takahashi et al., 2019; Orisaka et al., 2021; Takahashi and Ogiwara, 2023). Quite recently, cholecystokinin, instead of GnRH, was shown to induce the secretion of FSH in medaka (Uehara et al., 2023). E2 enters the circulatory system, and induces LH surge (Robker et al., 2018; Duffy et al., 2019; Takahashi et al., 2019; Orisaka et al., 2021; Takahashi and Ogiwara, 2023). LH surge activates mitogen-activated protein kinase (MAPK) cascades, and then the phosphorylation of ERK1/2 in antral follicles, which in turn activates multiple signaling cascades, including the phosphorylation of maturation-promoting factor (MPF, CDK1/CYB). Subsequently, MPF triggers GVBD, namely, oocyte maturation via activation of various factors (Robker et al., 2018; Duffy et al., 2019; Takahashi et al., 2019; Jessus et al., 2020; Takahashi and Ogiwara, 2023).

Unlike vertebrates, invertebrates lack glycoproteins homologous to FSH and LH, although an ortholog of another glycoprotein, thyrostimulin, which is not involved in follicular development, is conserved in various invertebrates as well as in vertebrates (Nakabayashi et al., 2002; Dos Santos et

al., 2009; Sun et al., 2010; Bassett et al., 2015; Wang et al., 2018; Yang et al., 2023). In non-ascidian invertebrates, several species-specific neuropeptides or compounds function as maturation-inducing factors: W/RPRPamide in jellyfish (Takeda et al., 2018), juvenile hormones in insects (Roy et al., 2018), and 1-methyladenine in starfish (Kanatani, 1985; Mita et al., 2009; Mita, 2023). Collectively, these findings show that oocyte maturation trigger molecules vary among phyla, but the intraovarian MAPK-MPF signaling cascade is conserved throughout metazoans (Jesus et al., 2020).

In early studies on oocyte maturation in ascidians, intracellular calcium ion mobilization and/or influx of calcium ions were believed to play a pivotal role in triggering GVBD in several marine invertebrates, including *Ciona* and other ascidians, although cAMP is responsible for GVBD in vertebrates and other invertebrates (Lambert, 2011; Deguchi et al., 2015). However, an ascidian GVBD inducer remained to be identified until 2019. Matsubara et al. (2019) demonstrated that the receptor for CiVP (CiVPR) is expressed predominantly in late stage II and early stage III (i.e., the preGVBD stages). Moreover, CiVPergic neurons in the cerebral ganglion were found to innervate the ovary by neuroanatomical analysis using the CiVP gene promoter-Kaede-transgenic *Ciona* (Kawada et al., 2021). These findings suggested that CiVP, secreted from the cerebral ganglion, participated in the regulation of GVBD. Indeed, treatment of *Ciona* pre-GVBD follicles with CiVP resulted in prompt induction of GVBD in the original in vitro follicle maturation/ovulation assays (Matsubara et al., 2019, 2020). Furthermore, CiVP was shown to activate ERK1/2, inducing the phosphorylation of *Ciona* MPF (Fig. 3). Eventually, phosphorylated MPF triggered GVBD (Matsubara et al., 2019; Satake, 2023) (Fig. 3). These findings are in good agreement with those of previous studies that showed that intracellular calcium ions are prerequisites for GVBD of ascidian oocytes (Lambert, 2011; Deguchi et al., 2015), given that CiVP induces intracellular calcium ion mobilization, but not production of cAMP, via the cognate receptor CiVPR (Kawada et al., 2008). Also of interest is that considerably fewer stage III follicles (post-GVBD follicles) were developed and, instead, more numerous stage I and stage II follicles were present in the ovaries of the TALEN-based CiVP-gene edited *Ciona* ovary, compared with the wild type (Kawada et al., 2021). Collectively, these studies have substantiated the molecular mechanism underlying *Ciona* oocyte maturation (Fig. 3).

These studies provided three major novel findings about *Ciona* follicle maturation. Firstly, GVBD is triggered by a VP family peptide in *Ciona*. Secondly, CiVP is a GVBD inducer in *Ciona*, whereas LH is responsible for induction of GVBD in vertebrates. This role of CiVP in the induction of GVBD is compatible with the findings that ascidians possess no orthologs of vertebrate pituitary gonadotropins FSH and LH (Dehal et al., 2002; Satake, 2023; Yang et al., 2023). Thirdly, in contrast with the species-specificity of a GVBD trigger, the basal MPF phosphorylation signaling cascade is evolutionarily conserved throughout metazoans, including ascidians. In combination, they support the conclusion that *Ciona* employs a VP family peptide as an inducer of oocyte maturation, and shares the intraovarian MPF phosphorylation-directed GVBD induction with other metazoans.

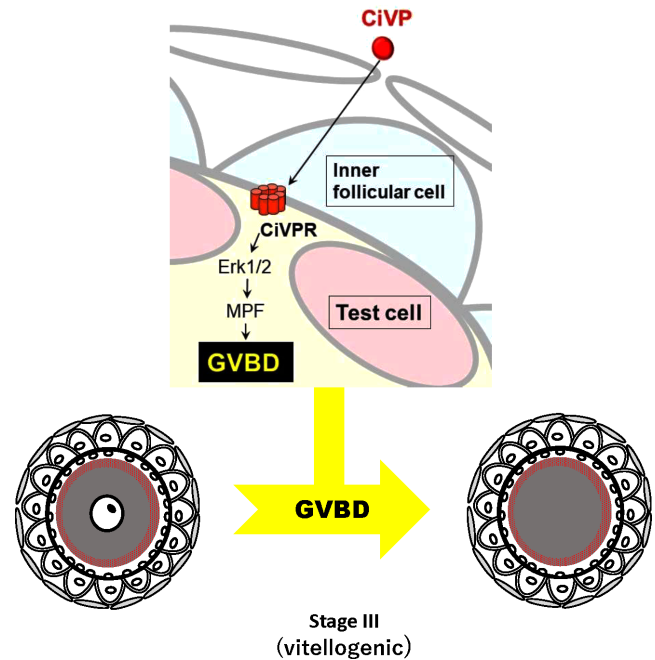


Fig. 3. Induction of oocyte maturation (GVBD). CiVP activates Erk1/2 in oocytes at stage III, and promotes GVBD via activation of MPF, leading to GVBD. Red dots indicate mitochondria.

Ovulation in *Ciona*

In vertebrates, LH surge also almost simultaneously induces ovulation as well as oocyte maturation. In mammals, LH stimulates multiple signaling pathways, including the cAMP-protein kinase A (PKA)-CREB, inositol triphosphate 3-intracellular calcium mobilization- ERK1/2, and PI3K/AKT pathways (Robker et al., 2018; Duffy et al., 2019). Activation of ERK1/2 results in the gene expression and secretion of epidermal growth factor (EGF)-like ligands in granulosa cells, leading to the formation of the cumulus oocyte complex (COC) that functions in the regulation of oocyte condition and fertilization (Robker et al., 2018; Duffy et al., 2019). These multiple signaling cascades also upregulate COX2, the synthase of prostaglandin H (PGH), which is a precursor of all PGs in granulosa cells (Robker et al., 2018; Duffy et al., 2019). PGE2, a dominant PG in granulosa cells, mediates follicular rupture and angiogenesis via its cognate receptor, EP2 (Robker et al., 2018; Duffy et al., 2019). Furthermore, the increase in progesterone synthesis by these signaling cascades up-regulates the gene expression of a matrix metalloprotease, ADAMTS-1, in granulosa cells, which is responsible for degradation of an extracellular matrix, versican, and release of COC from the follicle (Robker et al., 2018; Duffy et al., 2019). Integration of such complex molecular mechanisms is required for the drastic remodeling of follicles, leading to ovulation of fertile oocytes.

In teleosts, including medaka, LH surge has been shown to upregulate the cAMP-PKA-CREB pathway, leading to the gene expression of MMP15 and a PGE2 receptor, PTGER4b, via non-MAPK signaling pathways (Takahashi et al., 2019; Ogiwara et al., 2023; Takahashi and Ogiwara, 2023). Furthermore, MMP15 is responsible for extracellular matrix degradation, and PTGER4b is a prerequisite for intracellular actin filament reorganization; both processes eventually

induce ovulation (Takahashi et al., 2019; Ogiwara et al., 2023; Takahashi and Ogiwara, 2023).

However, neither the ovulation of mature *Ciona* follicles nor the relevant molecular mechanisms have been investigated. The ovulation of mature *Ciona* follicles was observed in vitro for the first time in 2019, and CiVP was identified as an ovulation inducer as well as a GVBD trigger in *Ciona* (Matsubara et al., 2019, 2020). CiVP-activated ERK1/2 also up-regulates the gene expression of the matrix metalloproteinase MMP2/9/13, and the degradation of the extracellular matrix by MMP2/9/13 results in ovulation (Matsubara et al., 2019). These studies verified that CiVP plays a crucial role in induction of ovulation as well as GVBD in *Ciona*, instead of LH in vertebrates (Fig. 4). Interestingly, vasotocin (VT), a non-mammalian vertebrate VP family peptide, was shown to induce oocyte maturation and ovulation in catfish (Joy and Chaube, 2015; Singh et al., 2021), although the balance in contribution to the ovulation process between VT and LH and its conservation in teleost species remain unclear. Notably, the ovulation process in *Ciona* has been shown to share some characteristics with both teleosts and mammals. For example, the activation of ERK1/2 leading to ovulation is conserved between *Ciona* and mammals (Matsubara et al., 2019; Kawada et al., 2021; Satake, 2023). In contrast, no evidence for the involvement of MMPs in ovulation in mammals has been provided. Instead, another matrix metalloprotease, ADAMTS-1, participates in the proteolysis of the extracellular matrix including versican during the ovulation process (Robker et al., 2018). Moreover, upregulation of the gene expression and the enzymatic activity of MMPs is a common signaling cascade during ovulation of *Ciona* and teleosts; however, ERK1/2 phosphorylation has not been detected in the ovulation pathway of teleosts (Matsubara et

al., 2019; Takahashi et al., 2019; Satake et al., 2023; Takahashi and Ogiwara, 2023). Taken together, these findings suggest that the *Ciona* intrafollicular pathway of ovulation induction appear to be a “hybrid” of mammalian and teleost ovulatory systems. In other words, ancestral chordates might have been endowed with a *Ciona*-like ovulation system, some parts of which have been conserved throughout vertebrates, while others have been newly acquired or diverged in each lineage of chordates.

The *Ciona* homolog of the vertebrate cholecystokinin (CCK), cionin (Johnsen and Rehfeld, 1990), has also been reported to elicit ovulation (Fig. 4). The cognate receptor, CioR2, is expressed specifically in inner follicular cells in early stage III follicles (Sekiguchi et al., 2012; Osugi et al., 2021). Moreover, cionin was found to increase the gene expression of receptor tyrosine kinase (RTK) signaling-related genes such as *rota*, *fcoll1*, and *gla3* (Osugi et al., 2021). Subsequently, RTK signaling upregulated gene expression, resulting in an increase in the enzymatic activity of MMP2/9/13, which eventually stimulates ovulation (Osugi et al., 2021; Kawada et al., 2022; Satake, 2023). These findings verified that cionin also serves as an ovulation inducer, and that *Ciona* possesses multiple distinct pathways for upregulation of MMPs leading to ovulation. Notably, unlike CiVP, cionin was found to induce only ovulation, but not GVBD (Osugi et al., 2021; Kawada et al., 2022; Satake, 2023). Furthermore, ERK1/2 phosphorylation, which leads to CiVP-directed GVBD and ovulation, was not implicated in the cionin-RTK-MMP cascade for ovulation (Fig. 4). In mammals, inhibition of RTK signaling resulted in suppression of ovulation (Bernard et al., 2016), although no endogenous stimulatory factor for the RTK signaling has yet been investigated. Moreover, CCK stimulated RTK signaling in non-ovarian cells (Zeng et al., 2020). These findings raise a question of whether CCK-RTK signaling is involved in vertebrate ovulation.

CONCLUSION AND PERSPECTIVES

As stated above, the main pathways for the peptidergic vitellogenic follicle growth, GVBD, and ovulation in *Ciona* (Fig. 5) have been elucidated over the course of the last 15 years. However, further investigations are required for better clarification of the net molecular mechanisms underlying *Ciona* follicle development. Firstly, a regulatory factor for the follicle growth from stage I to stage II has yet to be identified (Fig. 5). Moreover, both other *Ciona* orthologs of vertebrate peptides such as tunicate GnRHs (Adams et al., 2003; Sakai et al., 2017, 2020) and *Ciona*-specific peptides including CiLFs and CiYFVs (Kawada et al., 2011; Matsubara et al., 2016; Satake, 2023) are likely to participate in the regulation of follicle development, given that their cognate receptors have

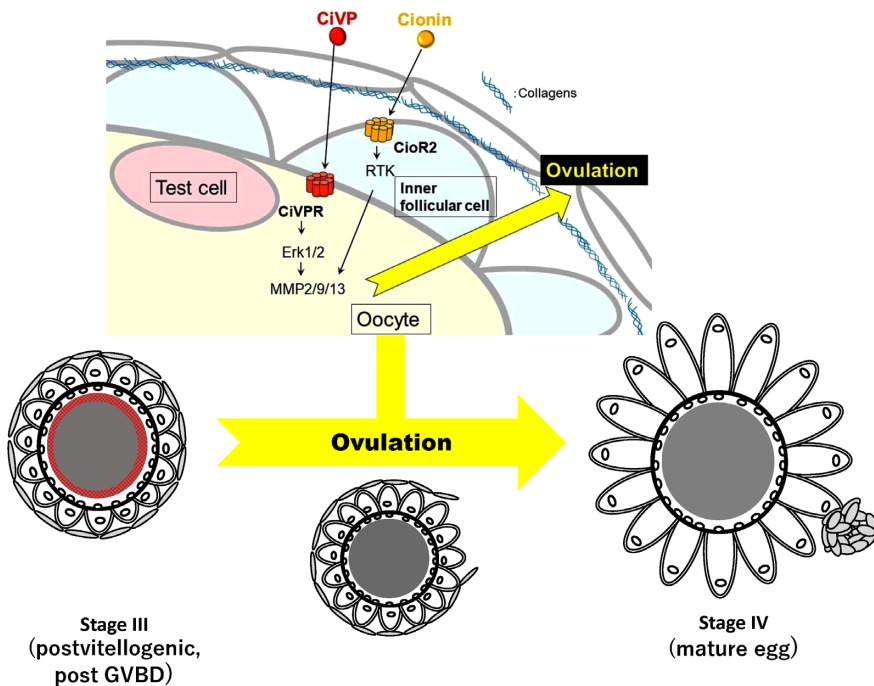


Fig. 4. Induction of ovulation. CiVP also upregulates MMP2/9/13 via activation of Erk1/2. Cionin also induces MMP2/9/13 expression via CioR2 in inner follicular cells and RTK signaling and subsequent ovulation in stage III follicles. Red dots indicate mitochondria.

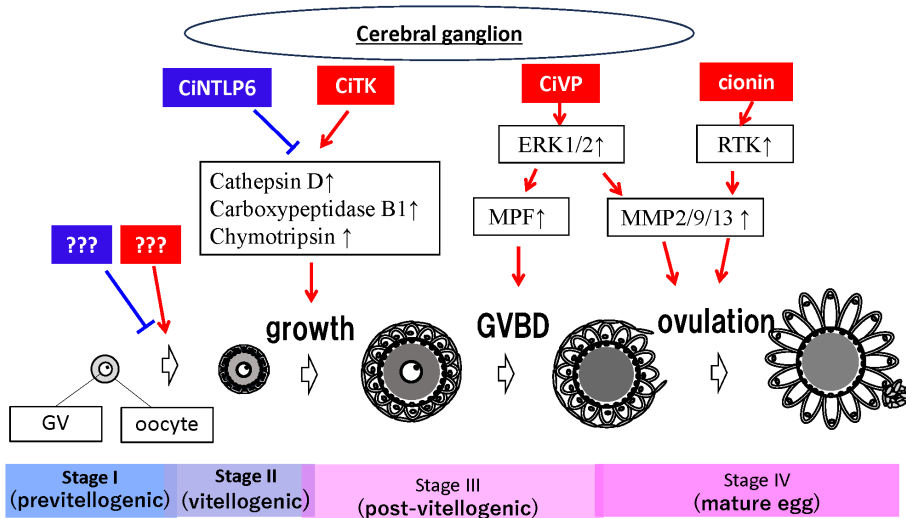


Fig. 5. Summary of neuroepidergic follicle development in *Ciona*. A regulatory factor for the growth from stage I to stage II has yet to be identified.

been shown to be expressed in the ovary or follicles (Sakai et al., 2010, 2012; Shiraishi et al., 2019). Of particular interest is a biological role of tGnRHs. In vertebrates, GnRHs enhance the synthesis and secretion of gonadotropins in the pituitary, including LH surge, which stimulates follicle growth, maturation, ovulation via the production of sex steroid hormones such as E2 and progesterone in the ovary (Edson et al., 2009; Richards and Pangas, 2010; Von Stetina and Orr-Weaver, 2011; Richards and Ascoli, 2018; Robker et al., 2018; Duffy et al., 2019). In contrast, as mentioned above, the *Ciona* genome lacks any sexual steroidogenic genes and pituitary hormone orthologs (Dehal et al., 2002). These findings, combined with the crucial phylogenetic position of ascidians as a sister group of the vertebrates, suggest a *Ciona*-specific biological role of GnRHs in the follicle development. Thus, the elucidation of the biological role of *Ciona* GnRHs will possibly explore an original function of GnRHs in common ancestral chordates before the acquisition of an HPG-axis prototype including the synthesis and secretion of gonadotropins and sex steroids. In keeping with these issues, secondly, verification of intrafollicular molecular mechanisms awaits further study. In vertebrates, a variety of secretory factors derived from oocytes, granulosa cells, and theca cells have been shown to participate in extensive or specific functions in the follicle development (Robker et al., 2018; Duffy et al., 2019; Takahashi et al., 2019; Takahashi and Ogiwara, 2023; Ogiwara et al., 2023). These findings and recently determined transcriptomes of the *Ciona* ovary (Kawada et al., 2017, 2021; Matsubara et al., 2021) suggest that a number of secretory factors from oocytes, test cells, or follicular cells are involved in the regulation of the follicle development in *Ciona*. Particularly, test cells are expected to secrete and/or produce such factors in a developmental stage-specific fashion, given that cellular structures and biological roles may vary at each follicle development stage (Mancuso, 1965; Wong et al., 2014); for example, secretory granules are present in test cells in stage-II follicles, but disappear at stage III (Mancuso, 1965). Characterization of the respective stage-specific oocyte- and accessory cell-derived regulatory factors is currently in progress. Such studies will

contribute a great deal to clarifying the entire follicle development in *Ciona* and the evolutionary history of the reproductive systems throughout chordates.

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COMPETING INTERESTS

The authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

HS, TO, and SM contributed to the conception and design of the manuscript. HS, TS, TK, TO, AS, TY, and MS wrote sections of the manuscript. HS, TO, and SM designed the figures. All authors approved the submitted version.

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