Ovarian Follicle Development in Ascidians

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

Source: Zoological Science, 41(1) : 60-67

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zs230054

ZOOLOGICAL SCIENCE **41**: 60–67 (2024)

[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 robusta*) 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 limited.

Ciona follicles are classified into four developmental stages: pre-vitellogenic (stage I), vitellogenic (stage II), postvitellogenic (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
doi:10.2108/zs230054

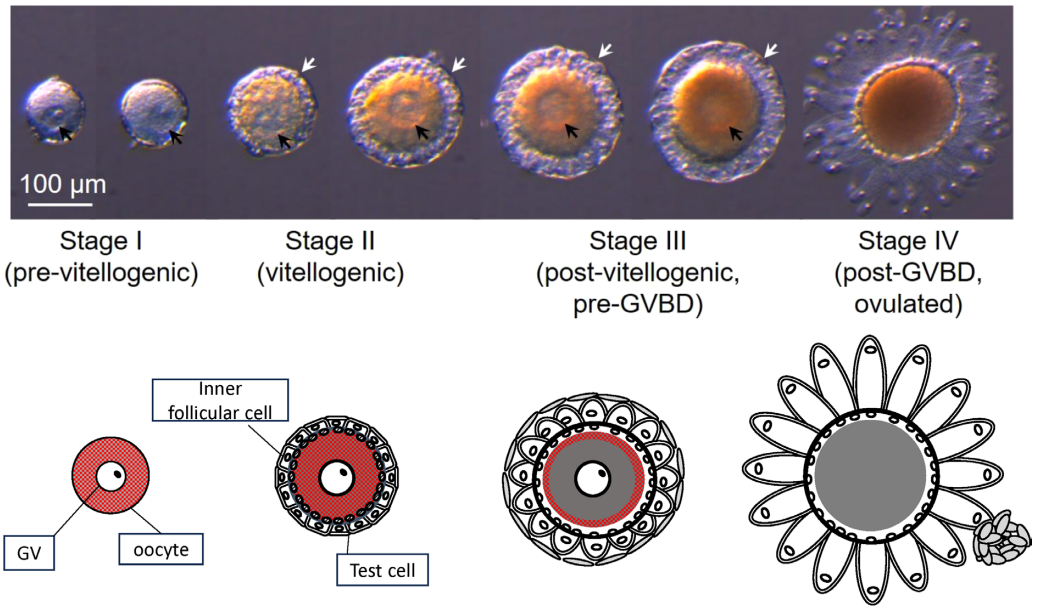

Fig. 1. Developmental stages of *Ciona* ovarian follicles. The ovary of adult ascidians contains numerous follicles at all developmental stages. Black and white arrows indicate germinal vesicle (GV) and outer follicular cell layers, respectively. Red dots indicate mitochondria. Permission has been obtained 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 triggered 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 (TGFβ) 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

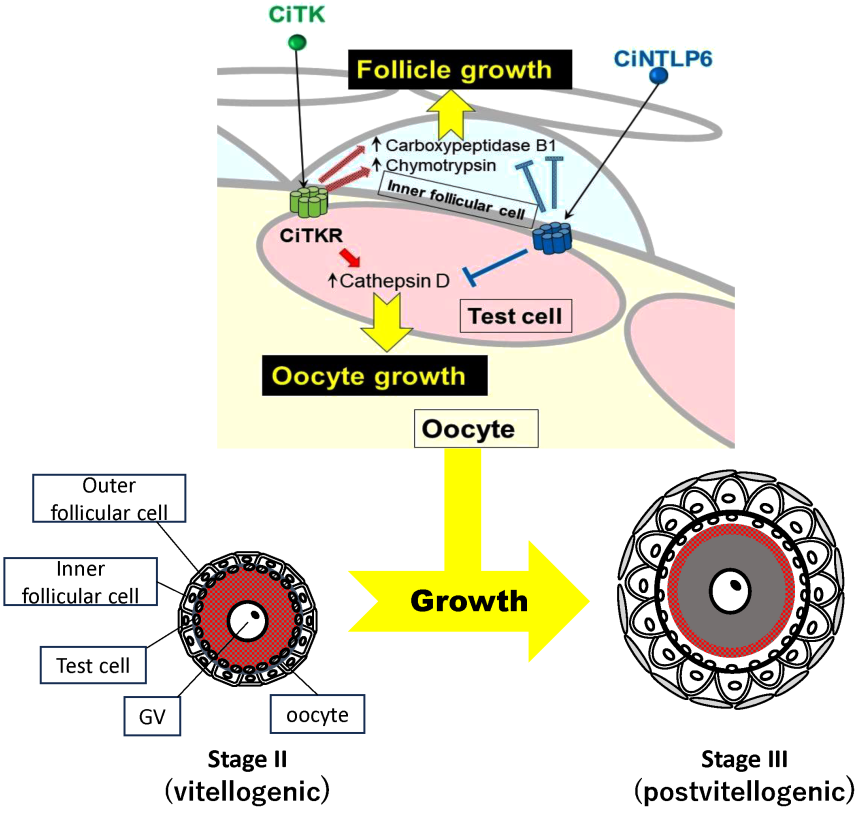

Fig. 2. Follicle growth from stage II to stage III. CiTK directly induces the gene expression of Cathepsin D in test cells. Moreover, CiTK secondarily upregulates the gene expression of Chymotrypsin and Carboxypeptidase B1 in inner follicular cells. Activation of these proteases is required for progression 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, CiNTLP-6 (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 CiNTLP6 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 17β-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 (Jessus 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.

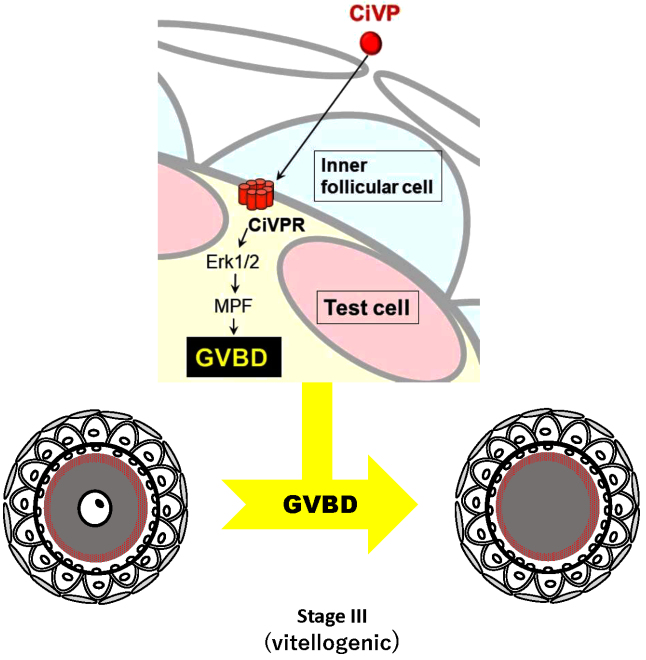

Fig. 3. Induction of oocyte maturation (GVBD). CiVP activates Erk1/2 in oocytes at stage III, and promotes GVBD via activation of MPF, leading to GVBD. Red dots indicate mitochondria.

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 *rora*, *fcol1*, 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.

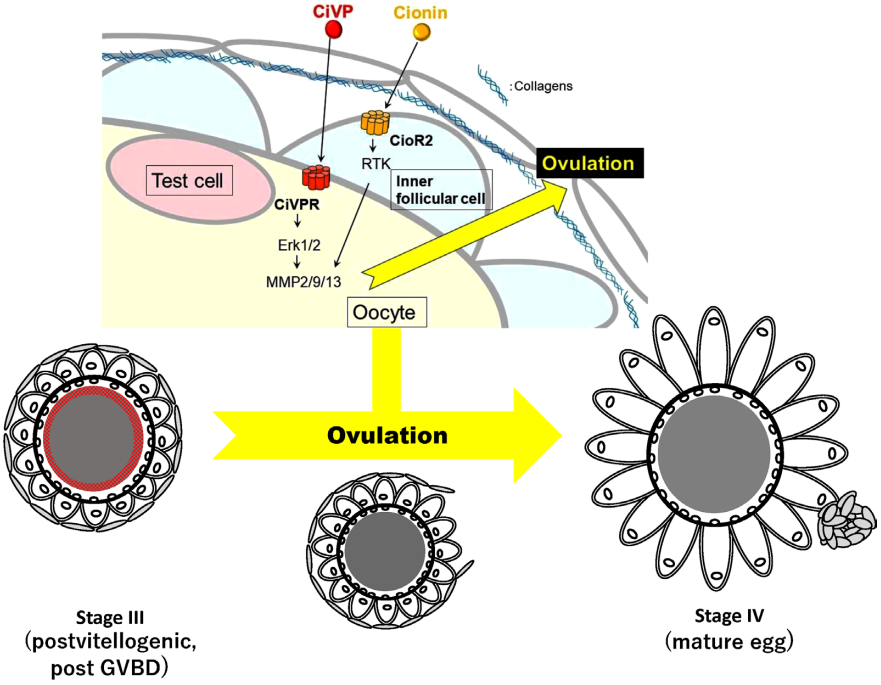

Fig. 4. Induction of ovulation. CiVP also upregulates MMP2/9/13 via activation of Erk1/2. Cionin also induces MMP2/9/13 expression via CioR2 in inner follicular cells and RTK signaling and subsequent ovulation in stage III follicles. Red dots indicate mitochondria.

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

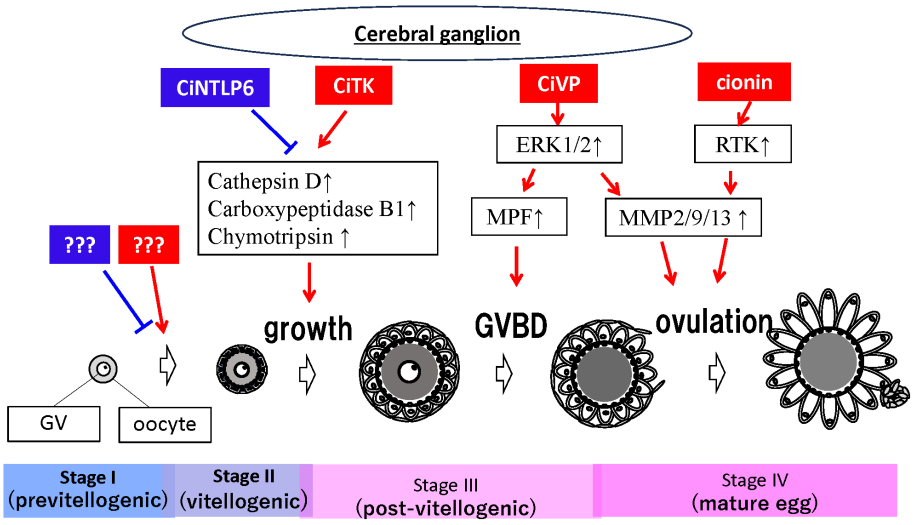

Fig. 5. Summary of neuropeptidergic follicle development in *Ciona*. A regulatory factor for the growth from stage I to stage II has yet to be identified.

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.

ACKNOWLEDGMENTS

This work was supported by grants from the Japan Society for the Promotion of Science (http://www.jsps.go.jp/english/index.html) to HS (JP19K06752).

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.

REFERENCES

Adams BA, Tello JA, Erchegyi J, Warby C, Hong DJ, Akinsanya KO, et al. (2003) Six novel gonadotropin-releasing hormones are encoded as triplets on each of two genes in the protochordate, *Ciona intestinalis*. Endocrinology 144: 1907–1919

Aoyama M, Kawada T, Fujie M, Hotta K, Sakai T, Sekiguchi T, et al. (2008) A novel biological role of tachykinins as an up-regulator of oocyte growth: identification of an evolutionary origin of tachykininergic functions in the ovary of the ascidian, *Ciona intestinalis*. Endocrinology 149: 4346–4356

Aoyama M, Kawada T, Satake H (2012) Localization and enzymatic activity profiles of the proteases responsible for tachykinin-directed oocyte growth in the protochordate, *Ciona intestinalis*. Peptides 34: 186–192

Azumi K, Sabau SV, Fujie M, Usami T, Koyanagi R, Kawashima T, et al. (2007) Gene expression profile during the life cycle of the urochordate *Ciona intestinalis*. Dev Biol 308: 572–582

Bassett JH, van der Spek A, Logan JG, Gogakos A, Bagchi-Chakraborty J, Williams AJ, et al. (2015) Thyrostimulin Regulates Osteoblastic Bone Formation During Early Skeletal Development. Endocrinology 156: 3098–3113

Bernard V, Bouilly J, Kramer P, Carré N, Schlumberger M, Visser JA, et al. (2016) The tyrosine kinase inhibitor sunitinib affects ovulation but not ovarian reserve in mouse: a preclinical study. PLOS ONE 11: e0152872

Blasco V, Pinto FM, Fernández-Atucha A, González-Ravina C, Fernández-Sánchez M, Candenas L (2020) Female infertility is associated with an altered expression of the neurokinin B/neurokinin B receptor and kisspeptin/kisspeptin receptor systems in ovarian granulosa and cumulus cells. Fertil Steril 114: 869–878

Blasco V, Pinto FM, Fernández-Atucha A, Dodd NP, Fernández-Sánchez M, Candenas L (2023) Female infertility is associated with an altered expression profile of different members of the tachykinin family in human granulosa cells. Reprod Sci 30: 258–269

Burighel P, Cloney RA (1997) *Urochordata ascidiacea*. In: "Microscopic Anatomy of Invertebrates, Vol 15" Ed by FW Harrison, Wiley-Liss, New York, pp 221–347

Cao C, Lemaire LA, Wang W, Yoon PH, Choi YA, Parsons LR, et al. (2019) Comprehensive single-cell transcriptome lineages of a

proto-vertebrate. Nature 571: 349–354
Carnevali O, Cionna C, Tosti L, Lubzens E, Maradonna F (2006) Role of cathepsins in ovarian follicle growth and maturation. Gen Comp Endocrinol 146: 195–203
Dalbies-Tran R, Cadoret V, Desmarchais A, Elis S, Maillard V, Monget P, et al. (2020) A comparative analysis of oocyte development in mammals. Cells 9: 1002
Deguchi R, Takeda N, Stricker SA (2015) Calcium signals and oocyte maturation in marine invertebrates. Int J Dev Biol 59: 271–280
Dehal P, Satou Y, Campbell RK, Chapman J, Degnan B, De Tomaso A, et al. (2002) The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. Science 298: 2157–2167
Delsuc F, Brinkmann H, Chourrout D, Philippe H (2006) Tunicates and not cephalochordates are the closest living relatives of vertebrates. Nature 439: 965–968
Denoeud F, Henriet S, Mungpakdee S, Aury JM, Da Silva C, Brinkmann H, et al. (2010) Plasticity of animal genome architecture unmasked by rapid evolution of a pelagic tunicate. Science 330: 1381–1385
Dos Santos S, Bardet C, Bertrand S, Escriva H, Habert D, Querat B (2009) Distinct expression patterns of glycoprotein hormone-alpha2 and -beta5 in a basal chordate suggest independent developmental functions. Endocrinology 150: 3815–3822
Dos Santos S, Mazan S, Venkatesh B, Cohen-Tannoudji J, Quérat B (2011) Emergence and evolution of the glycoprotein hormone and neurotrophin gene families in vertebrates. BMC Evol Biol 11: 332
Duffy DM, Ko C, Jo M, Brannstrom M, Curry TE (2019) Ovulation: Parallels with inflammatory processes. Endocr Rev 40: 369–416
Edson MA, Nagaraja AK, Matzuk MM (2009) The mammalian ovary from genesis to revelation. Endocr Rev 30: 624–712
García-Ortega J, Pinto FM, Fernández-Sánchez M, Prados N, Cejudo-Román A, Almeida TA, et al. (2014) Expression of neurokinin B/NK3 receptor and kisspeptin/KISS1 receptor in human granulosa cells. Hum Reprod 29: 2736–2746
Heyland A, Plachetzki D, Donelly E, Gunaratne D, Bobkova Y, Jacobson J, et al. (2012) Distinct expression patterns of glycoprotein hormone subunits in the lophotrochozoan Aplysia: implications for the evolution of neuroendocrine systems in animals. Endocrinology 153: 5440–5451
Horie R, Hazbun A, Chen K, Cao C, Levine M, Horie T (2018) Shared evolutionary origin of vertebrate neural crest and cranial placodes. Nature 560: 228–232
Imai KS, Hino K, Yagi K, Satoh N, Satou Y (2004) Gene expression profiles of transcription factors and signaling molecules in the ascidian embryo: towards a comprehensive understanding of gene networks. Development 131: 4047–4058
Imai KS, Levine M, Satoh N, Satou Y (2006) Regulatory blueprint for a chordate embryo. Science 312: 1183–1187
Imai KS, Daido Y, Kusakabe TG, Satou Y (2012) Cis-acting transcriptional repression establishes a sharp boundary in chordate embryos. Science 337: 964–967
Jakobsen RK, Ono S, Powers JC, DeLotto R (2005) Fluorescently labeled inhibitors detect localized serine protease activities in *Drosophila melanogaster* pole cells, embryos, and ovarian egg chambers. Histochem Cell Biol 123: 51–60
Jessus C, Munro C, Houliston E (2020) Managing the oocyte meiotic arrest – Lessons from frogs and jellyfish. Cells 9: 1150
Johnsen AH, Rehfeld JF (1990) Cionin: a disulfotyrosyl hybrid of cholecystokinin and gastrin from the neural ganglion of the protochordate *Ciona intestinalis*. J Biol Chem 265: 3054–3058
Joy KP, Chaube R (2015) Vasotocin--A new player in the control of oocyte maturation and ovulation in fish. Gen Comp Endocrinol 221: 54–63
Kanatani H (1985) Oocyte growth and maturation in starfish. In "Biology of Fertilization, Vol 1" Ed by CB Metz, A Monroy, Academic Press, New York, pp 119–140
Kato S, Tsurumaru S, Taga M, Yamane T, Shibata Y, Ohno K, et al. (2009) Neuronal peptides induce oocyte maturation and gamete spawning of sea cucumber, *Apostichopus japonicus*. Dev Biol 326: 169–176
Kawada T, Sekiguchi T, Itoh Y, Ogasawara M, Satake H (2008) Characterization of a novel vasopressin/oxytocin superfamily peptide and its receptor from an ascidian, *Ciona intestinalis*. Peptides 29: 1672–1678
Kawada T, Ogasawara M, Sekiguchi T, Aoyama M, Hotta K, Oka K, et al. (2011) Peptidomic analysis of the central nervous system of the protochordate, *Ciona intestinalis*: homologs and prototypes of vertebrate peptides and novel peptides. Endocrinology 152: 2416–2427
Kawada T, Shiraishi A, Aoyama M, Satake H (2017) Transcriptomes of the premature and mature ovaries of an ascidian, *Ciona intestinalis*. Front Endocrinol (Lausanne) 8: 88
Kawada T, Shiraishi A, Matsubara S, Hozumi A, Horie T, Sasakura Y, et al. (2021) Vasopressin promoter transgenic and vasopressin gene-edited ascidian, *Ciona intestinalis* Type A (*Ciona robusta*): Innervation, gene expression profiles, and phenotypes. Front Endocrinol (Lausanne) 12: 668564
Kawada T, Osugi T, Matsubara S, Sakai T, Shiraishi A, Yamamoto T, et al. (2022) Omics studies for the identification of ascidian peptides, cognate receptors, and their relevant roles in ovarian follicular development. Front Endocrinol (Lausanne). 13: 858885
Lambert CC (2011) Signaling pathways in ascidian oocyte maturation: the roles of cAMP/Epac, intracellular calcium levels and calmodulin kinase in regulating GVBD. Mol Reprod Dev 78: 726–733
Litscher ES, Qi H, Wassarman PM (1999) Mouse zona pellucida glycoproteins mZP2 and mZP3 undergo carboxy-terminal proteolytic processing in growing oocytes. Biochemistry 38: 12280–12287
Mancuso V (1965) An electron microscope study of the test cells and follicle cells of *Ciona intestinalis* during oogenesis. Acta Embryol Morphol Exp 8: 239–266
Matsubara S, Kawada T, Sakai T, Aoyama M, Osugi T, Shiraishi A, et al. (2016) The significance of *Ciona intestinalis* as a stem organism in integrative studies of functional evolution of the chordate endocrine, neuroendocrine, and nervous systems. Gen Comp Endocrinol 227: 101–108
Matsubara M, Shiraishi A, Osugi T, Kawada T, Satake H (2019) The regulation of oocyte maturation and ovulation in the closest sister group of vertebrates. eLife 8: e49062
Matsubara S, Shiraishi A, Osugi T, Kawada T, Satake H (2020) Fractionation of ovarian follicles and in vitro oocyte maturation and ovulation assay of *Ciona intestinalis* type A. Bio Protoc 10: e3577
Matsubara S, Osugi T, Shiraishi A, Wada A, Satake H (2021) Comparative analysis of transcriptomic profiles among ascidians, zebrafish, and mice: Insights from tissue-specific gene expression. PLOS ONE 16: e0254308
Mita M (2023) Relaxin-like gonad-stimulating peptides in Asteroidea. Biomolecules 13: 781
Mita M, Yoshikuni M, Ohno K, Shibata Y, Paul-Prasanth B, Pitchayawasin S, et al. (2009) A relaxin-like peptide purified from radial nerves induces oocyte maturation and ovulation in the starfish, *Asterina pectinifera*. Proc Natl Acad Sci USA 106: 9507–9512
Nakabayashi K, Matsumi H, Bhalla A, Bae J, Mosselman S, Hsu SY, et al. (2002) Thyrostimulin, a heterodimer of two new human glycoprotein hormone subunits, activates the thyroid-stimulating hormone receptor. J Clin Invest 109: 1445–1452
Ogiwara K, Fujimori C, Takahashi T (2023) The PGE_2/Ptger4b

pathway regulates ovulation by inducing intracellular actin cytoskeleton rearrangement via the Rho/Rock pathway in the granulosa cells of periovulatory follicles in the teleost medaka. Mol Cell Endocrinol 560: 111816

Oishi A, Gengyo-Ando K, Mitani S, Mohri-Shiomi A, Kimura KD, Ishihara T, et al. (2009) FLR-2, the glycoprotein hormone alpha subunit, is involved in the neural control of intestinal functions in *Caenorhabditis elegans*. Genes Cells 14: 1141–1154

Orisaka M, Miyazaki Y, Shirafuji A, Tamamura C, Tsuyoshi H, Tsang BK, et al. (2021) The role of pituitary gonadotropins and intra-ovarian regulators in follicle development: A mini-review. Reprod Med Biol. 20: 169–175

Osugi T, Sasakura Y, Satake H (2017) The nervous system of the adult ascidian *Ciona intestinalis* Type A (*Ciona robusta*): Insights from transgenic animal models. PLOS ONE 12: e0180227

Osugi T, Sasakura Y, Satake H (2020) The ventral peptidergic system of the adult ascidian *Ciona robusta* (*Ciona intestinalis* Type A) insights from a transgenic animal model. Sci Rep 10: 1892

Osugi T, Miyasaka N, Shiraishi A, Matsubara S, Satake H (2021) Cionin, a vertebrate cholecystokinin/gastrin homolog, induces ovulation in the ascidian *Ciona intestinalis* type A. Sci Rep 11: 10911

Prodon F, Chenevert J, Sardet C (2006) Establishment of animal-vegetal polarity during maturation in ascidian oocytes. Dev Biol 290: 297–311

Richards JS, Ascoli M (2018) Endocrine, paracrine, and autocrine signaling pathways that regulate ovulation. Trends Endocrinol Metab 29: 313–325

Richards JS, Pangas SA (2010) The ovary: basic biology and clinical implications. J Clin Invest 120: 963–972

Robker RL, Hennebold JD, Russell DL (2018) Coordination of ovulation and oocyte maturation: A good egg at the right time. Endocrinology 159: 3209–3218

Rocco DA, Paluzzi JV (2020) Expression profiling, downstream signaling, and inter-subunit interactions of GPA2/GPB5 in the adult mosquito *Aedes aegypti*. Front Endocrinol (Lausanne) 11: 158

Roy S, Saha TT, Zou Z, Raikhel AS (2018) Regulatory pathways controlling female insect reproduction. Annu Rev Entomol 63: 489–511

Sakairi K, Shirai H (1991) Possible MIS production by follicle cells in spontaneous oocyte maturation of the ascidian, *Halocynthia roretzi*. Dev Growth Differ 33: 155–162

Sasakura Y, Awazu S, Chiba S, Satoh N (2003) Germ-line transgenesis of the Tc1/ mariner superfamily transposon Minos in *Ciona intestinalis*. Proc Natl Acad Sci USA 100: 7726–7730

Sasakura Y, Horie T (2023) Improved genome editing in the ascidian *Ciona* with CRISPR/Cas9 and TALEN. Methods Mol Biol 2637: 375–388

Sasakura Y, Yoshida K, Treen N (2017) Genome editing of the ascidian *Ciona intestinalis* with TALE Nuclease. Methods Mol Biol 1630: 235–245

Satake H (2023) Kobayashi Award 2021: Neuropeptides, receptors, and follicle development in the ascidian, *Ciona intestinalis* Type A: New clues to the evolution of chordate neuropeptidergic systems from biological niches. Gen Comp Endocrinol 337: 114262

Satake H, Ogasawara M, Kawada T, Masuda K, Aoyama M, Minakata H, et al. (2004) Tachykinin and tachykinin receptor of an ascidian, *Ciona intestinalis*: evolutionary origin of the vertebrate tachykinin family. J Biol Chem 279: 53798–53805

Satoh N (2003) The ascidian tadpole larva: comparative molecular development and genomics. Nat Rev Genet 4: 285–295

Satoh N, Rokhsar D, Nishikawa T (2014) Chordate evolution and the three-phylum system. Proc Biol Sci 281: 20141729

Satou Y, Kawashima T, Shoguchi E, Nakayama A, Satoh N (2005) An integrated database of the ascidian, *Ciona intestinalis*: Towards functional genomics. Zool Sci 22: 837–843

Satou Y, Mineta K, Ogasawara M, Sasakura Y, Shoguchi E, Ueno K, et al. (2008) Improved genome assembly and evidence-based global gene model set for the chordate *Ciona intestinalis*: new insight into intron and operon populations. Genome Biol 9: R152

Satou Y, Nakamura R, Yu D, Yoshida R, Hamada M, Fujie M, et al. (2019) A nearly complete genome of *Ciona intestinalis* Type A (*C. robusta*) reveals the contribution of inversion to chromosomal evolution in the genus *Ciona*. Genome Biol Evol 11: 3144–3157

Sekiguchi T, Ogasawara M, Satake H (2012) Molecular and functional characterization of cionin receptors in the ascidian, *Ciona intestinalis*: the evolutionary origin of the vertebrate cholecystokinin/gastrin family. J Endocrinol 213: 99–106

Shiraishi A, Okuda T, Miyasaka N, Osugi T, Okuno Y, Inoue J, et al. (2019) Repertoires of G protein-coupled receptors for *Ciona*-specific neuropeptides. Proc Natl Acad Sci USA 116: 7847–7856

Singh V, Chaube R, Joy KP (2021) Vasotocin stimulates maturation-inducing hormone, oocyte maturation and ovulation in the catfish *Heteropneustes fossilis*: Evidence for a preferential calcium involvement. Theriogenology 167: 51–60

Sun SC, Hsu PJ, Wu FJ, Li SH, Lu CH, Luo CW (2010) Thyrostimulin, but not thyroid-stimulating hormone (TSH), acts as a paracrine regulator to activate the TSH receptor in mammalian ovary. J Biol Chem 285: 3758–3765

Takagi-Sawada M, Someno T, Hoshi M, Sawada H (1989) Inhibition of starfish oocyte maturation by leupeptin analogs, potent trypsin inhibitors. Dev Biol 133: 609–612

Takahashi T, Ogiwara K (2023) cAMP signaling in ovarian physiology in teleosts: A review. Cell Signal 101: 110499

Takahashi T, Hagiwara A, Ogiwara K (2019) Follicle rupture during ovulation with an emphasis on recent progress in fish models. Reproduction 157: R1–R13

Takeda N, Kon Y, Quiroga Artigas G, Lapébie P, Barreau C, Koizumi O, et al. (2018) Identification of jellyfish neuropeptides that act directly as oocyte maturation-inducing hormones. Development 145: dev156786

Tanaka E, Takagi-Sawada M, Sawada H (2000) Enzymatic properties of the proteasome purified from starfish oocytes and its catalytic subunits involved in oocyte maturation. Comp Biochem Physiol C 125: 215–223

Uehara SK, Nishiike Y, Maeda K, Karigo T, Kuraku S, Okubo K, et al. (2023) Identification of the FSH-RH, the other gonadotropin-releasing hormone. BioRxiv 2023.05.26.542428

Von Stetina JR, Orr-Weaver TL (2011) Developmental control of oocyte maturation and egg activation in metazoan models. Cold Spring Harb Perspect Biol 3: a005553

Wang P, Liu S, Yang Q, Liu Z, Zhang S (2018) Functional characterization of thyrostimulin in amphioxus suggests an ancestral origin of the TH signaling pathway. Endocrinology 159: 3536–3548

Wong GW, Zhuo L, Kimata K, Lam BK, Satoh N, Stevens RL (2014) Ancient origin of mast cells. Biochem Biophys Res Commun 451: 314–318

Yang LK, Zhang J, Liu D, Han TY, Qin QS, Wang AQ, et al. (2023) Ancestral glycoprotein hormone and its cognate receptor present in primitive chordate ascidian: Molecular identification and functional characterization. Int J Biol Macromol 229: 401–412

Zeng Q, Ou L, Wang W, Guo DY (2020) Gastrin, cholecystokinin, signaling, and biological activities in cellular processes. Front Endocrinol (Lausanne) 11: 112

(Received June 24, 2023 / Accepted July 20, 2023 /
Published online September 15, 2023)