INTRODUCTION

Xenopus laevis has been employed for many years to study a variety of developmental processes, such as gametogenesis, embryogenesis, morphogenesis, neurogenesis, organogenesis, metamorphosis, regeneration, and reproduction (for monographs and/or protocols see: Ref. 1-5). Its whole-genome sequence has been published recently, and in combination with that of Xenopus tropicalis, a diploidic subspecies in Xenopus, whose whole-genome sequence was reported earlier, genome-wide genetic approaches, such as transcriptome analysis and genome editing studies are now actively pursued in a number of research projects. In particular, eggs and oocytes have been considered to be one of the ideal experimental models to study oocyte maturation and egg fertilization, because of their large size, relative abundance, and clearly defined progression from oogenesis within the ovary to initiation of zygotic development.
in response to sperm-induced activation. A growing body of knowledge indicates that many of the molecular and cellular features defining *X. laevis* oogenesis, maturation, ovulation, and fertilization may also be identical with those in mammalian species, including human, further suggesting physiological relevance of *X. laevis* as an excellent model for egg/oocyte’s life cycle from cradle to grave (Figure 1).

## 2 | OVARY AND OOCYGENESIS

A sexually mature, that is adult, female animal of *X. laevis* contains tens of thousands of oocytes in the ovary, in which asynchronous oogenesis of oocytes at stages I to VI occurs at any given time. Growth of ovarian/follicular oocytes, which are surrounded by the follicle envelope that consists of several layers such as epithelial/ theca layer, granulose cell layer, and vitelline envelope, from stage I (smallest and partly transparent, Figure 1) to stage VI (largest, Figure 1), requires more than eight months. In the laboratory, oogenesis is influenced by a variety of environmental factors, such as crowding (more than 1.5 L per animal), feeding (twice a week), water exchange (more than twice a week), hormonal stimulation of animals (once in 3-4 months). Upon hormonal stimulation of ovulation, many but not all of the stage VI oocytes are released from ovary (see “maturation and ovulation”). The remaining oocytes of stages I-V undergo accelerated progression of oogenesis. At the same time, the stage VI oocytes remaining in the ovary after ovulation either await the next hormonal stimulation or undergo follicular atresia, a kind of apoptotic process that eliminates oocytes from the ovary.

At present, molecular mechanisms underlying oogenesis and follicular atresia in frogs are not well studied. One main reason for this is the lack of an in vitro reconstruction system for analyzing oogenesis of *X. laevis*. In vitro culture of ovary and/or oocytes that allows the oocytes to progress from stage I-VI has not been established. Admittedly, oogenesis in the ovary is the most mysterious, unknown phenomenon of oocyte biology in *X. laevis*. Fabbri et al.10 and Larose et al.11 highlighted recent trends and achievements in the field of in vitro oogenesis or more widely gametogenesis in mammals, with use of ovarian tissue culture or stem cell technology.

## 3 | OVULATION AND MATURATION

All oocytes that reside and grow in the ovary of *X. laevis* are arrested, as in many other vertebrate species, in prophase of the first meiotic division (Pro-I) irrespective of their oogenesis stages (I-VI). The ovarian oocytes are incompetent for fertilization and are called “immature” oocytes. The immature oocytes acquire competence for fertilization in the process of meiotic maturation, where the full-grown stage VI oocytes exit the meiotic arrest, progress through meiotic cell cycle, and arrest again in metaphase of the second meiotic division (Meta-II). In *X. laevis*, progesterone (PG) is suggested to be a major physiological effector that promotes oocyte maturation, and this steroid hormone is also used to induce *Xenopus* oocyte maturation in vitro. The follicle responses to hormonal stimulation that produce fertilization-competent, that is, “mature” follicle layer-free oocytes are collectively termed “ovulation.”

In *X. laevis*, acquisition of fertilization competence or oocyte maturation consists of three major events: cytoplasmic, membrane, and extracellular maturation. Cytoplasmic maturation (eg, activation of maturation-promoting factor and MAPK cascade) and membrane maturation (eg, preparation of sperm-interacting machinery for egg activation, for detail see below) occur simultaneously during ovulation,12,13 while extracellular maturation (eg, transformation of the vitelline envelope and acquisition of jelly layer) occurs during passage of the oocytes through oviduct.14,15 These events have been well documented, and several reconstruction approaches have been developed for their in vitro studies.13 On the other hand, molecular details of oocyte liberation from ovarian follicles and release into the oviduct, as well as coordination of these processes with oocyte maturation, have not been well studied. Some in vitro ovulation models have been developed in mammals16-18 and the medaka fish *Oryzias latipes*.19-21 In frogs, in vitro maturation and ovulation of *Rana pipens* and *Rana dybowskii* oocytes was observed in the ovarian fragments and isolated follicles treated with pituitary extracts. In these studies, however, the experimental treatments failed to induce follicular rupture.

Under these circumstances, we have developed recently an in vitro ovulation model that reconstitutes both maturation and follicle rupture. The two processes can be synchronously recapitulated by treating isolated follicular oocytes with PG and collagenase, a matrix metalloproteinase (MMP).23 Inhibition of the MAPK pathway in these settings suppresses both germinal vesicle breakdown (GVBD) and follicular rupture, whereas inhibition of MMP activity delays follicular rupture without affecting GVBD, demonstrating for the first time that both MAPK and MMP are involved in oocyte release from ovarian follicles in frogs. Further study will be needed to elucidate where (ie, oocyte and/or follicular layer) and what kind of MAPK and MMP activities, including their molecular identity and enzymatic regulation, are necessary for oocyte ovulation and maturation.

## 4 | FERTILIZATION AND ACTIVATION OF DEVELOPMENT

Fertilization involves specific recognition and interaction of two gamete cells, egg, and sperm, which is followed by a series of processes called egg activation and initiation of zygotic development. Two key questions of fertilization studies in several model organisms are as follows: how does the sperm-egg interaction and fusion occur (for reviews: Ref. 24-26), and how does the fertilized egg initiate a transient elevation in intracellular calcium concentration (Ca2+ transient) that is necessary for successful activation of embryonic development (for reviews: Ref. 27-33).

For more than 25 years, eggs of *X. laevis* have been an excellent model to study sperm-induced intracellular signaling mediated by the Src tyrosine kinase, (34, for reviews: Ref. 13,32,35,36).
In *X. laevis*, a single transmembrane protein named uroplakin IIIa and ganglioside GM1, both of which localize to low-density, detergent-insoluble fractions of the egg/oocyte plasma membrane (so-called membrane microdomains or rafts), are considered as the candidate molecules for egg/oocyte surface receptor that engages the sperm-initiated intracellular signal by a Src-dependent transduction mechanism. In addition, several components in sperm, such as sperm glycoprotein named SGP, a membrane-matrix metalloproteinase 2, and tryptic protease, have been suggested to act as egg-interacting partners at fertilization.

The molecular details of the sperm-egg interaction and subsequent egg activation in *X. laevis* are different from those in mammalian species. In mice, gamete interaction and fusion are shown to be mediated by the sperm protein IZUMO1 and the egg/oocyte proteins CD9 and Juno; sperm-dependent activation of eggs is mediated by phospholipase C that is introduced from fertilizing sperm into the egg cytoplasm. **The latter mechanism is named as “sperm factor mechanism” while the mechanism inferred in *X. laevis* is named as “membrane contact mechanism.”** The difference between the mechanisms of egg fertilization and activation in frogs and mammals is intriguing, considering that cellular and molecular details of oogenesis, ovulation, and maturation (eg, regulation of meiosis; coordinated actions of MPF, MAPK, and CSF) in mouse and *X. laevis* are similar, as studied so far.

In this connection, it is worth noting that Liao et al reported that uroplakins and a Src-related tyrosine kinase play conserved roles in fertilization of the mouse and *X. laevis*. Uroplakins are a family of uroplakin proteins (UPs) that consists of two tetraspanins UP1a and UP1b, and three single transmembrane proteins UPII, UPIIIa, and UPIIIb in the mouse and other vertebrates such as cows and frogs. The mouse UPIIIa corresponds to *X. laevis* UPIII. In oocytes, UPIIIa and some other UPs co-localize with CD9 protein on the cell surface and in the multivesicular body-derived exosomes, and an intracellular tyrosine residue of UPIIIa is phosphorylated in a Fyn tyrosine kinase-dependent manner at fertilization. Antibody-blocking and knockout experiments also support the hypothesis that egg/oocyte-associated UPIIIa and some other UPs are involved in successful mouse fertilization. The similar experiments involving knockouts of UPIII and UP1b, a partner of UPIII for its membrane localization, in the diploidic frog species *Xenopus tropicalis* are currently under way in our laboratory.

**FIGURE 1** Life cycle events of oocytes and eggs in *Xenopus laevis*: from its cradle (oogenesis in ovary) through ovulation and maturation to physiological (sperm-dependent initiation of zygotic development, ie, fertilization) or pathological ending (spontaneous activation-mediated death by apoptosis or overactivation)

In *X. laevis*, maturing eggs relocate from the ovaries into the coelomic body cavity, pass through the oviduct, and accumulate in the uterus before being released out into the water where fertilization occurs. Unfertilized eggs die by a well-defined apoptotic process within 48-72 hours after spawning. The hallmark features of apoptosis, such as cytochrome c release, caspase activation, apoptotic nuclear morphology, increase of the ADP/ATP ratio, ATP depletion, have been observed in apoptotic frog eggs. It has been demonstrated that extracellular signal (eg, sperm)-independent exit from metaphase II arrest, which we term “spontaneous activation,” precedes apoptosis. Our more recent studies demonstrated that numerous cytoplasmic mRNAs are robustly degraded in apoptotic *Xenopus* eggs. Such global decay of mRNA is mediated by endonucleolyis and becomes evident in the eggs after meiotic exit at the time of cytochrome c release. In addition, we found that activity of senescence-associated β-galactosidase was elevated in the course of PG-induced aging and apoptosis in...
X laevis oocytes, although its physiological impact on apoptotic oocyte is not known. Apoptotic events were also observed in cell-free extracts of Xenopus eggs, propagating through the egg cytoplasm as apoptosis trigger waves. Notably, only interphase extracts, but not extracts arrested in metaphase, are susceptible to apoptosis.

Eggs from other vertebrate (eg, fish and mammals) and invertebrate species (eg, sea urchin and starfish) have also been found to die by apoptosis if they are not fertilized (for review: Ref. 67). It is conceivable that in viviparous species, such as mammals with internal fertilization, apoptosis removes ovulated unfertilized eggs without a pronounced inflammatory response, thereby supporting optimal body function. Indeed, fragmentation of ovulated murine oocytes and calcium-triggered degradation of rat eggs are well-known examples of caspase-dependent apoptosis.

However, the occurrence of apoptosis in the eggs of oviparous species with external fertilization (eg, fish, frog, sea urchin, and starfish) raises a question of its physiological significance. In this connection, it is important to note that a number of mature Metaphase-II-arrested eggs are retained in the genital tract of Xenopus frogs over several days following hormone-induced ovulation. All of the apoptotic events observed in unfertilized spawned eggs were also observed in the eggs retained in the genital tract, suggesting that the same apoptotic program unfolds in externally spawned and internally retained eggs. Thus, apoptosis of post-meiotic eggs, both deposited and retained, accompanies ovulation in frogs. The observation of egg apoptosis in the frog genital tract points to its physiological relevance. Although it has little significance in the case of water-deposited eggs, it may be important for elimination of the mature, overripe eggs retained in the frog body after ovulation.

As mentioned earlier, egg apoptosis in X laevis is preceded by spontaneous activation that occurs at 16-24 hours after PG administration, as judged by progressive decolorization of the egg surface and dephosphorylation of MAPK, a component of CSF that maintains the egg/oocyte at Meta-II, followed by caspase activation. At present, physiological inducers of spontaneous egg activation, mediated, presumably, by an elevation of intracellular calcium concentration, remain unidentified. It was suggested that oxidative stress might act as the initiator for a cascade of events that lead to expedited aging and deterioration of postovulatory oocytes. Our previous study demonstrated that hydrogen peroxide initiates tyrosine phosphorylation and elevates intracellular calcium resulting in Src kinase-dependent egg activation. The study also reported that prolonged treatment with hydrogen peroxide led to excessive cortical contraction and egg swelling. Interestingly, these morphological changes are also evident, albeit with a low frequency, in naturally ovulated and/or externally spawned eggs. This dramatic process, which we term "overactivation," rapidly progresses to its completion within 60 minutes in both spontaneously induced and hydrogen peroxide-treated eggs. At present, the intracellular events associated with spontaneous egg overactivation remain unexplored. On the other hand, we have demonstrated, using hydrogen peroxide-induced overactivation as a model process, that lipofuscin accumulation, decrease of soluble cytoplasmic protein content, and depletion of intracellular ATP occur in overactivated Xenopus eggs.

6 | PERSPECTIVES

Use of model animals is of central importance in designing appropriately and investigating effectively scientific research in biology and medicine. Among the commonly employed model animals, X laevis, an anuran amphibian species, has been widely used over the past several dozens of years and achieved unprecedented attention, especially in the fields of embryology and developmental biology, because of its unique experimental accessibility, cost-benefit performance, and close evolutionary relationship with mammals, as a tetrapod among the vertebrate species. In addition to the classical use, X laevis has also been employed in many modern approaches of life sciences, such as high-throughput DNA sequencing, genome editing, proteomics, pharmacological screening. The Xenopus frog was used to study fundamental biological and disease mechanisms not only in experimental settings but also in newly emerging theoretical and computational platforms.

Xenopus laevis and other amphibians are also known as good bioindicators of environmental pollution and changes in human activity and climate (Pecl et al 2017), because of their high sensitivity to chemical compounds in freshwater. This kind of research direction, for example, environmental study and toxicology, is especially important for development of better predictive tools for environmental protection of endangered anuran and some other wild species.

Biochemical alterations in such primary pesticide targets as cholinesterases and molecular biomarkers with a relation to transmembrane signal transduction and transcriptional regulation, whose implication in the oocyte maturation, fertilization, and embryogenesis is evident, have gained increasing attention. On the other hand, naturally occurring climatic phenomena and/or any kind of human activity-driven redistribution of chemical species (inorganic objects) and biological species (all living things) at regional and global scales greatly affect ecosystem functioning, human well-being, and the dynamics of climate change itself. Considerations of chemical diversity and biodiversity redistribution are critical yet largely lacking in most worldwide mitigation and adaptation strategies, such as the United Nation's Sustainable Development Goals (http://www.un.org/sustainabledevelopment/). With these concerns in mind, we would like to keep in the further research using oocytes and eggs of X laevis to the principle "leave no oocyte behind."

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CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest regarding the publication of this article.

DISCLOSURE
This article does not contain any studies with human subjects performed by the any of the authors.

ORCID
Ken-ichi Sato https://orcid.org/0000-0003-4808-5210

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