MINI REVIEW

Reproductive Medicine and Biology *Open Access* WILEY

Toward the understanding of biology of oocyte life cycle in *Xenopus Laevis*: No oocytes left behind

Ken-ichi Sato [iD] | Alexander A. Tokmakov

Laboratory of Cell Signaling and Development, Department of Industrial Life Sciences, Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Japan

Correspondence
Ken-ichi Sato, Laboratory of Cell Signaling and Development, Department of Industrial Life Sciences, Faculty of Life Sciences, Kyoto Sangyo University, Kamigamo-Motoyama, Kita-ku, Kyoto 603-8555, Japan.
Email: kksato@cc.kyoto-su.ac.jp

Abstract

Background: For the past more than 25 years, we have been focusing on the developmental and reproductive biology of the female gametes, oocytes, and eggs, of the African clawed frog *Xenopus laevis*.

Methods: The events associated with the life cycle of these cells can be classified into the four main categories: first, oogenesis and cell growth in the ovary during the first meiotic arrest; second, maturation and ovulation that occur simultaneously and result in the acquisition of fertilization competence and the second meiotic arrest; third, fertilization, that is sperm-induced transition from egg to zygote; and fourth, egg death after spontaneous activation in the absence of fertilizing sperm.

Main findings: Our studies have demonstrated that signal transduction system involving tyrosine kinase Src and other oocyte/egg membrane-associated molecules such as uroplakin III and some other cytoplasmic proteins such as mitogen-activated protein kinase (MAPK) play important roles for successful ovulation, maturation, fertilization, and initiation of embryonic development.

Conclusion: We summarize recent advances in understanding cellular and molecular mechanisms underlying life cycle events of the oocytes and eggs. Our further intention is to discuss and predict potentially promising impact of the recent findings on the challenges facing reproductive biology and medicine, as well as societal contexts.

KEYWORDS
fertilization, oocyte maturation, oogenesis, signal transduction, *Xenopus laevis*

1 | 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,[6] and in combination with that of *Xenopus tropicalis*, a

diploidic subspecies in *Xenopus*, whose whole-genome sequence was reported earlier,[7] 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 OOGENESIS

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.[8,9] 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,[12] 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 mammals [16-18] 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 rapture.[22]

Under these circumstances, we have developed recently an in vitro ovulation model that reconstitutes both maturation and follicle rapture. 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 rapture, whereas inhibition of MMP activity delays follicular rapture 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 (Ca^{2+} 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).

OOGENESIS
From Stage I to VI

Within ovary

First Meiotic Arrest
at Pro-I

OVULATION and **MATURATION**
From Immature to Mature

Exit from Ovary to Oviduct

Second Meiotic Arrest
at Meta-II

APOPTOSIS

FERTILIZATION

OVERACTIVATION

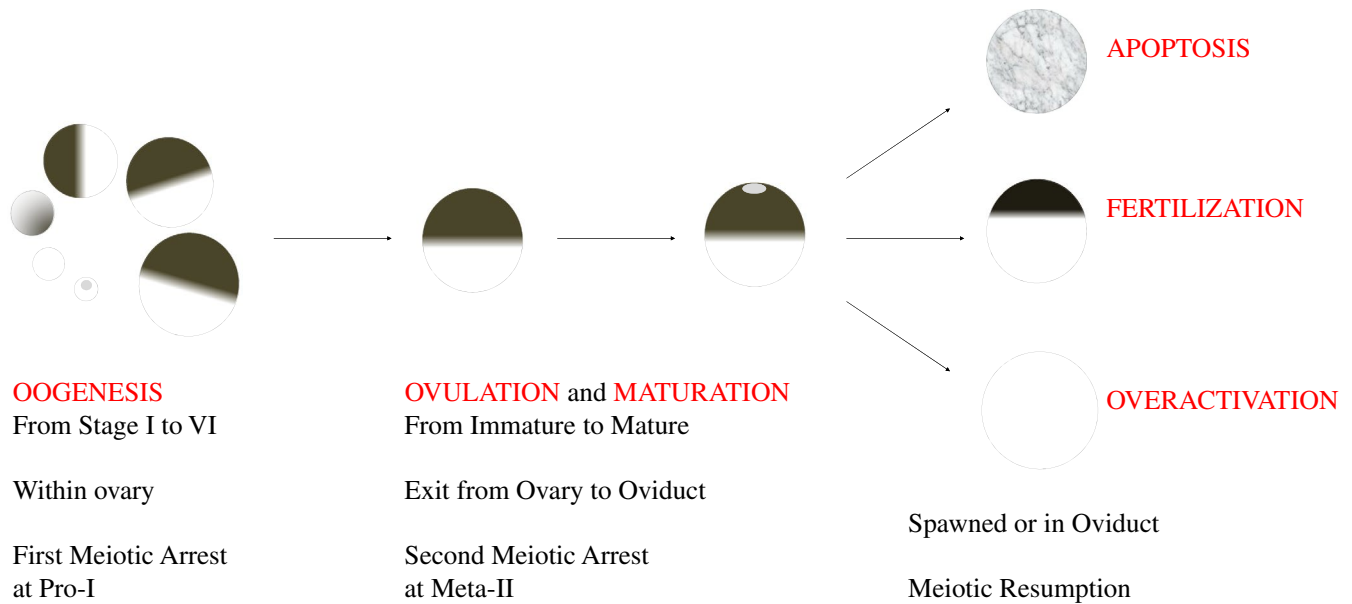Spawned or in Oviduct

Meiotic Resumption

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*, a single transmembrane protein named uroplakin IIIa [37-39] and ganglioside GM1,[40,41] both of which localize to low-density, detergent-insoluble fractions of the egg/oocyte plasma membrane (so-called membrane microdomains or rafts),[42,43] 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,[44] a membrane-matrix metalloproteinase 2,[40] and tryptic protease,[37,45] 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 [46] and the egg/oocyte proteins CD9[47-50] and Juno[51]; sperm-dependent activation of eggs is mediated by phospholipase Cζ that is introduced from fertilizing sperm into the egg cytoplasm.[52-54] 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[55] 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 UPIa and UPIb, and three single transmembrane proteins UPII, UPIIIa, and UPIIIb in the mouse and other vertebrates such as cows and frogs.[56-58] 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 UPIb, a partner of UPIII for its membrane localization,[38,41,59] in the diploidic frog species *Xenopus tropicalis* are currently under way in our laboratory.

5 | SPONTANEOUS OOCYTE DEATH

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.[60,61] 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.[61] Our more recent studies demonstrated that numerous cytoplasmic mRNAs are robustly degraded in apoptotic *Xenopus* eggs.[62] Such global decay of mRNA is mediated by endonucleolysis 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.[63] Apoptotic events were also observed in cell-free extracts of *Xenopus* eggs,[64] propagating through the egg cytoplasm as apoptosis trigger waves.[65] Notably, only interphase extracts, but not extracts arrested in metaphase, are susceptible to apoptosis.[66]

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[68] and calcium-triggered degradation of rat eggs[69] 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 Meta-II-arrested eggs are retained in the genital tract of *Xenopus* frogs over several days following hormone-induced ovulation.[60,70] 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.[70] 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.[61,67] 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.[71] Our previous study demonstrated that hydrogen peroxide initiates tyrosine phosphorylation and elevates intracellular calcium resulting in Src kinase-dependent egg activation.[72] 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.[73] 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.[73]

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.[74,75] 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 [76] 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.[76] On the other hand, naturally occurring climatic phenomena and/or any kind of human activity-driven redistribution of chemical species (inorganic objects) [78] and biological species (all living things)[77] 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."

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

REFERENCES

1. Green SL, ed. *The Laboratory* Xenopus *sp.* (1st edn). Boca Raton, FL:CRC Press; 2009.
2. Hoppler S, Vize PD, eds. *Xenopus Protocols Post-Genomic Approaches, Part of the Methods in Molecular Biology book series* (MIMB, vol. 917), Totowa, NJ:Humana Press; 2012.
3. Kay BK, Peng B, eds. *Xenopus laevis: Practical Uses in Cell and Molecular Biology. Methods in Cell Biology* (vol. 36). Cambridge, MA:Academic Press; 1991.
4. Liu JX, ed. *Xenopus Protocols: Cell Biology and Signal Transduction. Part of the Methods in Molecular Biology Book Series* (MIMB, vol. 322). Totowa, NJ:Humana Press; 2006.
5. Vleminckx K, ed. *Xenopus Methods and Protocols*. Berlin, Germany:Springer; 2018.
6. Session AM, Uno Y, Kwon T, et al. Genome evolution in the allotetraploid frog *Xenopus laevis*. *Nature*. 2016;538(7625):336-343.
7. Hellsten U, Harland RM, Gilchrist MJ, et al. The genome of the Western clawed frog *Xenopus tropicalis*. *Science*. 2010;328(5978):633-636.
8. Dumont JN. Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J Morphol*. 1972;136(2):153-179.
9. Rasar MA, Hammes SR. The physiology of the *Xenopus laevis* ovary. *Methods Mol Biol*. 2006;322:17-30.
10. Fabbri R, Zamboni C, Vicenti R, Macciocca M, Paradisi R, Seracchioli R. Update on oogenesis in vitro. *Minerva Ginecol*. 2018;70(5):588-608.
11. Larose H, Shami AN, Abbott H, Manske G, Lei L, Hammoud SS. Gametogenesis: a journey from inception to conception. *Curr Top Dev Biol*. 2019;132:257-310.
12. Ferrell Jr JE *Xenopus* oocyte maturation: new lessons from a good egg. *BioEssays*. 1999;21(10):833-842.
13. Sato K. Transmembrane signal transduction in oocyte maturation and fertilization: focusing on *Xenopus laevis* as a model animal. *Int J Mol Sci*. 2014;16(1):114-134.
14. Gerton GL. Biochemical studies of the envelope transformations in *Xenopus laevis* eggs. *Adv Exp Med Biol*. 1986;207:133-149.
15. Ueda Y, Kubo H, Iwao Y. Characterization of the acrosome reaction-inducing substance in *Xenopus* (ARISX) secreted from the oviductal pars recta onto the vitelline envelope. *Dev Biol*. 2002;264(1):289-298.
16. Curry Jr TE, Smith MF. Impact of extracellular matrix remodeling on ovulation and the folliculo-luteal transition. *Semin Reprod Med*. 2006;24(4):228-241.
17. Robker RL, Russell DL, Yoshioka S, et al. Ovulation: a multi-gene, multi-step process. *Steroids*. 2000;65(10–11):559-570.
18. Skory RM, Xu Y, Shea LD, Woodruff TK. Engineering the ovarian cycle using in vitro follicle culture. *Hum Reprod*. 2015;30(6):1386-1395.
19. Ogiwara K, Takano N, Shinohara M, Murakami M, Takahashi T. Gelatinase A and membrane-type matrix metalloproteinases 1 and 2 are responsible for follicle rupture during ovulation in the medaka. *Proc Natl Acad Sci U S A*. 2005;102(24):8442-8447.
20. Ogiwara K, Ikeda T, Takahashi T. A new in vitro ovulation model for medaka based on whole ovary culture. *Zoolog Sci*. 2010;27(9):762-767.
21. Takahashi T, Fujimori C, Hagiwara A, Ogiwara K. Recent advances in the understanding of teleost medaka ovulation: the roles of proteases and prostaglandins. *Zoolog Sci*. 2013;30(4):239-247.
22. Subtelny S, Smith LD, Ecker RE. (1968) Maturation of ovarian frog eggs without ovulation. *J Exp Zool*. 1968;168(1):39-47.
23. Tokmakov AA, Matsumoto Y, Isobe T, Sato KI. In vitro reconstruction of *Xenopus* oocyte ovulation. *Int J Mol Sci*. 2019;20(19):E4766.
24. Bhakta HH, Refai FH, Avella MA. The molecular mechanisms mediating mammalian fertilization. *Development*. 2019;146(15):dev176966.
25. Okabe M. The cell biology of mammalian fertilization. *Development*. 2013;140(22):4471-4479.
26. Okabe M. Sperm-egg interaction and fertilization: past, present, and future. *Biol Reprod*. 2018;99(1):134-146.
27. Ciapa B, Chiri S. Egg activation: upstream of the fertilization calcium signal. *Biol Cell*. 2000;92(3–4):215-233.
28. Ducibella T, Fissore R. The roles of Ca^{2+}, downstream protein kinases, and oscillatory signaling in regulating fertilization and the activation of development. *Dev Biol*. 2008;315(2):257-279.
29. Kinsey WH. SRC-family tyrosine kinases in oogenesis, oocyte maturation and fertilization: an evolutionary perspective. *Adv Exp Med Biol*. 2014;759:33-56.
30. Nuccitelli R. How do sperm activate eggs? *Curr Top Dev Biol*. 1991;25:1-16.
31. Parrington J, Arnoult C, Fissore RA. The eggstraordinary story of how life begins. *Mol Reprod Dev*. 2019;86(1):4-19.
32. Runft LL, Jaffe LA, Mehlmann LM. Egg activation at fertilization: where it all begins. *Dev Biol*. 2002;245(2):237-254.
33. Santella L, Lim D, Moccia F. Calcium and fertilization: the beginning of life. *Trends Biochem Sci*. 2004;29(8):400-408.
34. Sato K, Tokmakov AA, Iwasaki T, Fukami Y. Tyrosine kinase-dependent activation of phospholipase Cgamma is required for calcium transient in *Xenopus* egg fertilization. *Dev Biol*. 2000;224(2):453-469.
35. Sato K, Tokmakov AA, Fukami Y. Fertilization signalling and protein-tyrosine kinases. *Comp Biochem Physiol B Biochem Mol Biol*. 2000b;126(2):129-148.
36. Sato K, Fukami Y, Stith BJ. Signal transduction pathways leading to Ca^{2+} release in a vertebrate model system: lessons from *Xenopus* eggs. *Semin Cell Dev Biol*. 2006;17(2):285-292.
37. Mahbub Hasan AK, Sato K, Sakakibara K, et al. Uroplakin III, a novel Src substrate in *Xenopus* egg rafts, is a target for sperm protease essential for fertilization. *Dev Biol*. 2005;286(2):483-492.
38. Mahbub Hasan AK, Hashimoto A, Maekawa Y, et al. The egg membrane microdomain-associated uroplakin III-Src system becomes functional during oocyte maturation and is required for bidirectional gamete signaling at fertilization in *Xenopus laevis*. *Development*. 2014;141(8):1705-1714.
39. Sakakibara K, Sato K, Yoshino K, et al. Molecular identification and characterization of *Xenopus* egg uroplakin III, an egg raft-associated transmembrane protein that is tyrosine-phosphorylated upon fertilization. *J Biol Chem*. 2005;280(15):15029-15037.
40. Iwao Y, Shiga K, Shiroshita A, et al. The need of MMP-2 on the sperm surface for *Xenopus* fertilization: its role in a fast electrical block to polyspermy. *Mech Dev*. 2014;134:80-95.
41. Mahbub Hasan AK, Ou Z, Sakakibara K, et al. Characterization of *Xenopus* egg membrane microdomains containing uroplakin Ib/III complex: roles of their molecular interactions for subcellular localization and signal transduction. *Genes Cells*. 2007;12(2):251-267.

42. Hasan AK, Fukami Y, Sato K. Gamete membrane microdomains and their associated molecules in fertilization signaling. *Mol Reprod Dev.* 2011;78(10–11):814-830.

43. Sato K, Iwasaki T, Ogawa K, Konishi M, Tokmakov AA, Fukami Y. Low density detergent-insoluble membrane of *Xenopus* eggs: subcellular microdomain for tyrosine kinase signaling in fertilization. *Development.* 2002;129(4):885-896.

44. Nagai K, Ishida T, Hashimoto T, et al. The Sperm-surface glycoprotein, SGP, is necessary for fertilization in the frog, Xenopus laevis. *Dev Growth Differ.* 2009;51(5):499-510.

45. Mizote A, Okamoto S, Iwao Y. (1999) Activation of *Xenopus* eggs by proteases: possible involvement of a sperm protease in fertilization. *Dev Biol.* 1999;208(1):79-92.

46. Inoue N, Ikawa M, Isotani A, Okabe M. The immunoglobulin superfamily protein Izumo is required for sperm to fuse with eggs. *Nature.* 2005;434(7030):234-238.

47. Kaji K, Oda S, Shikano T, et al. The gamete fusion process is defective in eggs of Cd9-deficient mice. *Nat Genet.* 2000;24(3):279-282.

48. Le Naour F, Rubinstein E, Jasmin C, Prenant M, Boucheix C. Severely reduced female fertility in CD9-deficient mice. *Science.* 2000;287(5451):319-321.

49. Miyado K, Yamada G, Yamada S, et al. Requirement of CD9 on the egg plasma membrane for fertilization. *Science.* 2000;287(5451):321-324.

50. Miyado K, Yoshida K, Yamagata K, et al. The fusing ability of sperm is bestowed by CD9-containing vesicles released from eggs in mice. *Proc Natl Acad Sci U S A.* 2008;105(35):12921-12926.

51. Bianchi E, Doe B, Goulding D, Wright GJ. Juno is the egg Izumo receptor and is essential for mammalian fertilization. *Nature.* 2014;508(7497):483-487.

52. Hachem A, Godwin J, Ruas M, et al. PLCζ is the physiological trigger of the Ca^{2+} oscillations that induce embryogenesis in mammals but conception can occur in its absence. *Development.* 2017;144(16):2914-2924.

53. Nozawa K, Satouh Y, Fujimoto T, Oji A, Ikawa M. Sperm-borne phospholipase C zeta-1 ensures monospermic fertilization in mice. *Sci Rep.* 2018;8(1):1315.

54. Saunders CM, Larman MG, Parrington J, et al. PLC zeta: a sperm-specific trigger of Ca^{2+} oscillations in eggs and embryo development. *Development.* 2002;129(15):3533-3544.

55. Liao Y, Chang HC, Liang FX, et al. Uroplakins play conserved roles in egg fertilization and acquired additional urothelial functions during mammalian divergence. *Mol Biol Cell.* 2018;29(26):3128-3143.

56. Garcia-España A, Chung PJ, Zhao X, et al. Origin of the tetraspanin uroplakins and their co-evolution with associated proteins: implications for uroplakin structure and function. *Mol Phylogenet Evol.* 2006;41(2):355-367.

57. Khandelwal P, Abraham SN, Apodaca G. Cell biology and physiology of the uroepithelium. *Am J Physiol Renal Physiol.* 2009;297(6):F1 477-F1501.

58. Wu XR, Kong XP, Pellicer A, Kreibich G, Sun TT. Uroplakins in urothelial biology, function, and disease. *Kidney Int.* 2009;75(11):1153-1165.

59. Tu L, Sun TT, Kreibich G. Specific heterodimer formation is a prerequisite for uroplakins to exit from the endoplasmic reticulum. *Mol Biol Cell.* 2002;13(12):4221-4230.

60. Du Pasquier D, Dupré A, Jessus C. Unfertilized *Xenopus* eggs die by Bad-dependent apoptosis under the control of Cdk1 and JNK. *PLoS ONE.* 2011;6(8):e23672.

61. Tokmakov AA, Iguchi S, Iwasaki T, Fukami Y. Unfertilized frog eggs die by apoptosis following meiotic exit. *BMC Cell Biol.* 2011;23(12):56.

62. Tokmakov AA, Iguchi S, Iwasaki T, Fukami Y, Sato KI. Global decay of mRNA is a hallmark of apoptosis in aging *Xenopus* eggs. *RNA Biol.* 2017;14(3):339-346.

63. Tokmakov AA, Sato KI. Activity and intracellular localization of senescence-associated β-galactosidase in aging *Xenopus* oocytes and eggs. *Exp Gerontol.* 2019;119:157-167.

64. Newmeyer DD, Farschon DM, Reed JC. Cell-free apoptosis in *Xenopus* egg extracts: inhibition by Bcl-2 and requirement for an organelle fraction enriched in mitochondria. *Cell.* 1994;79(2):353-364.

65. Cheng X, Ferrell Jr JE. Apoptosis propagates through the cytoplasm as trigger waves. *Science.* 2018;361(6402):607-612.

66. Tashker JS, Olson M, Kornbluth S. Post-cytochrome C protection from apoptosis conferred by a MAPK pathway in *Xenopus* egg extracts. *Mol Biol Cell.* 2002;13(2):393-401.

67. Tokmakov AA, Sato KI, Stefanov VE. Postovulatory cell death: why eggs die via apoptosis in biological species with external fertilization. *J Reprod Dev.* 2018;64(1):1-6.

68. Perez GI, Tao XJ, Tilly JL. Fragmentation and death (a.k.a. apoptosis) of ovulated oocytes. *Mol Hum Reprod.* 1999;5(5):414-420.

69. Tripathi A, Chaube SK. High cytosolic free calcium level signals apoptosis through mitochondria-caspase mediated pathway in rat eggs cultured in vitro. *Apoptosis.* 2012;17(5):439-448.

70. Iguchi S, Iwasaki T, Fukami Y, Tokmakov AA. Unlaid *Xenopus* eggs degrade by apoptosis in the genital tract. *BMC Cell Biol.* 2013;3(14):11.

71. Lord T, Aitken RJ. Oxidative stress and ageing of the post-ovulatory oocyte. *Reproduction.* 2013;146(6):R217-R227.

72. Sato K, Ogawa K, Tokmakov AA, Iwasaki T, Fukami Y. Hydrogen peroxide induces Src family tyrosine kinase-dependent activation of *Xenopus* eggs. *Dev Growth Differ.* 2001;43(1):55-72.

73. Tokmakov AA, Awamura M, Sato KI. Biochemical hallmarks of oxidative stress-induced overactivation of *Xenopus* eggs. *Biomed Res Int.* 2019;2019:7180540.

74. Khokha MK *Xenopus* white papers and resources: folding functional genomics and genetics into the frog. *Genesis.* 2012;50(3):133-142.

75. Sater AK, Moody SA. Using *Xenopus* to understand human disease and developmental disorders. *Genesis.* 2017;55(1–2).

76. Venturino A, Rosenbaum E, Caballero de Castro A, et al. Biomarkers of effect in toads and frogs. *Biomarkers.* 2003;8(3–4):167-186.

77. Pecl GT, Araújo MB, Bell JD, et al. Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science.* 2017;355(6332):eaai9214.

78. Mahaffy PG, Matlin SA, Holme TA, MacKellar J. Systems thinking for education about the molecular basis of sustainability. *Nature Sustainability.* 2019;2:362-370.