2

The Physiology of the *Xenopus laevis* Ovary

Melissa A. Rasar and Stephen R. Hammes

Summary

Xenopus laevis has been used for many decades to study oocyte development and maturation. The *Xenopus* oocytes' large size, relative abundance, and clearly defined progression of physical characteristics from oogonia to eggs make them ideal for studying oogenesis. In addition, the ability of steroids to trigger *Xenopus* oocyte maturation in vitro has resulted in their extensive use for the study of the complexities of meiosis. Interestingly, steroid-induced maturation of *Xenopus* oocytes occurs completely independent of transcription; thus, this process serves as one of the few biologically relevant models of nongenomic steroid-mediated signaling. Finally, *Xenopus* oocytes appear to play a critical role in ovarian steroidogenesis, suggesting that the *Xenopus* ovary may serve as a novel system for studying steroidogenesis. Evidence indicates that many of the features defining *Xenopus laevis* oogenesis and maturation might also be occurring in mammals, further emphasizing the strength and relevance of *Xenopus laevis* as a model for ovarian development and function.

Key Words: Maturation; oocyte; ovary; steroidogenesis; vitellogenesis; *Xenopus*.

1. Anatomy of the Ovary

The ovaries are considered the largest organs in the adult female frog, filling a large portion of the abdominal cavity and contributing to nearly 15% of the total frog weight during breeding ***(1)***. The two ovaries resemble large transparent sacks subdivided into multiple lobes (~24 lobes per ovary), each containing hundreds of oocytes at all stages of development (**Fig. 1**). Unlike in mammals, the relatively large frog oocytes make up the majority of the ovarian volume. Furthermore, these oocytes contribute to important ovarian physiologic processes, such as steroidogenesis. The large size and easy accessibility of amphibian oocytes also make them useful tools for studying meiosis; thus, *Xenopus* oocytes have long served as an important model for examining the cell cycle.

Although they rest in the abdominal cavity, the ovaries are actually fixed to the retroperitoneum. They are attached to the kidneys and are surrounded by an epithelium

From: *Methods in Molecular Biology, vol. 322:* Xenopus *Protocols: Cell Biology and Signal Transduction*
Edited by: X. J. Liu © Humana Press Inc., Totowa, NJ

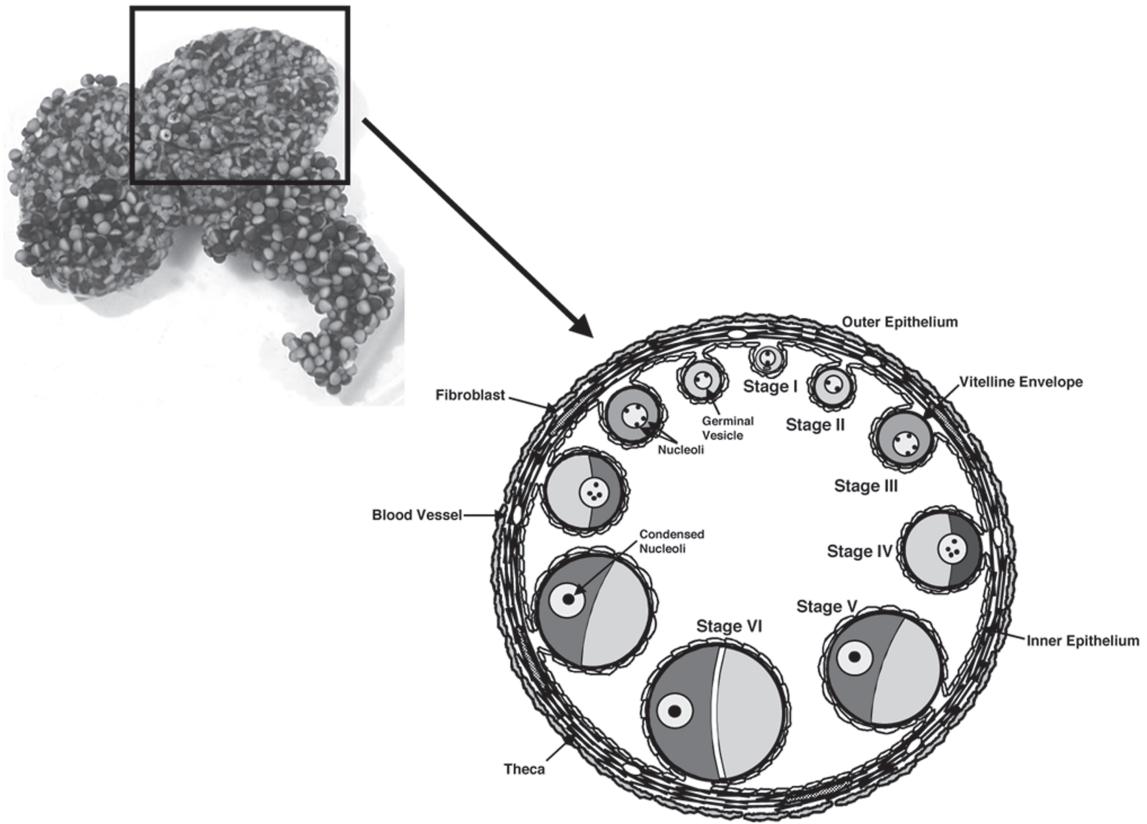

Fig. 1. Photograph and schematic representation of the *Xenopus* ovary and oogenesis. An individual ovary consists of approx 24 lobes (upper left). Every lobe is made up of hundreds of follicles, each of which contains an individual oocyte at any stage of development (schematic on the right). *See* text for details.

of ciliated cells derived from embryonic primordial medullary tissue that is continuous with the visceral peritoneum ***(2)***. This epithelium is made up of a single layer of flat cells covering an inner thecal layer. The thecal layer consists of extracellular matrix surrounding blood vessels and fibroblasts and covers another layer of cells that constitute the inner epithelium.

At the start of oogenesis, oocytes are located within the thecal layer. As they grow, they push the inner epithelium centrally to form bulges that represent individual follicles. The individual oocytes are surrounded by a layer of flattened cells that keep them isolated from the thecal layer, with each oocyte and associated cells representing an independent follicle.

During follicular development, the surrounding layer of follicle cells becomes separated from the oocytes by an acellular layer of mucopolysaccharide material called the vitelline membrane or zona radiata. Although the origin of this membrane is not certain, some evidence suggests that it might be secreted by the follicle cells themselves during secondary follicle growth ***(3)***.

As the follicles continue to grow, the follicle cells extend small projections that cross this vitelline membrane to contact the oocyte surface and form gap junctions. These gap junctions are felt to play important roles in the communication between oocytes and surrounding follicle cells and may be involved in essential processes such as yolk accumulation and the regulation of meiosis ***(4)***. A schematic representation of ovarian and oocyte anatomy is depicted in **Fig. 1**.

2. Anatomy of the Developed Oocyte

Oocytes that are in their final stage of development (stage VI) have completed oogenesis and are competent for ovulation and subsequent fertilization ***(3,5)***. These oocytes are approx 1.3 mm in diameter, with a volume of approx 1 μL. They have a well-defined brown animal pole (containing the majority of the nucleus) and a weakly pigmented vegetal hemisphere (containing the majority of the yolk platelets). Between the two hemispheres is a thin, unpigmented ring called the equatorial band or belt.

The nucleus, or germinal vesicle, of a stage VI oocyte is quite large relative to a somatic cell nucleus (~10^5 times the size), and holds multiple extranucleoli containing large amounts of ribosomal ribonucleic acid (RNA). The DNA within the nucleus has replicated and is arrested in the diplotene stage of prophase I. The germinal vesicle is surrounded by a nuclear envelope containing large pores, which are likely important for the transport of molecules such as messenger RNA (mRNA) between the cytoplasm and the nucleus ***(6)***.

The cytoplasm of the oocyte is also unique in developed oocytes, with 80% of the cytoplasmic proteins being yolk proteins stored in the yolk platelets ***(7)***. These consist of lipoproteins and glycoproteins that will serve as nutritive stores for the developing embryo. The process of yolk protein accumulation is known as *vitellogenesis* and is described in **Subheading 4.** The cytoplasm also contains a rich store of maternal mRNAs and ribosomes.

Although protein synthesis is very robust during oocyte development, only 2% of ribosomes are active, and only 20% of mRNAs are actively translated ***(8,9)***.

The remaining ribosomes are stored for later function. Inactive mRNAs are stored in ribonuclear protein (RNP) complexes, and specific transcripts may be compartmentalized within the oocyte. Furthermore, these dormant mRNAs may be incompletely polyadenylated, thus rendering them inactive until the resumption of meiosis, when the polyadenylation pattern of mRNA is dramatically altered ***(10,11)***.

One last important feature of the developed oocyte is its cortex. The cortex consists of a cytokeratin shell extending a few micrometers from the membrane toward the cytoplasm and appears to play an important role in maintaining the structural integrity of the oocyte ***(3)***. The melanin-containing pigmented granules are present in this region and are more concentrated around the animal pole of the oocyte. In addition, the microvilli from surrounding follicle cells extend through the cortex, allowing communication between the oocyte and surrounding cells. Finally, the cortex is surrounded by the plasma membrane, which directly contacts the vitelline envelope of the follicle.

3. Oogenesis

Oogenesis refers to the transformation of oogonia to oocytes. Oogenesis begins in the embryo approx 3 wk after fertilization, when the embryonic gonads start to become sexually differentiated. During this time, the primordial germ cells begin to multiply to form primary oogonia. This growth is still quite slow and asynchronous. By 4 wk, the oogonia have begun to couple together, resulting in the formation of secondary oogonia. During this time, meiosis begins. Meiosis continues to the diplotene stage of prophase I, at which point the oocyte is arrested until just prior to ovulation. During this protracted diplotene stage, the oocytes grow considerably, increasing their volume by well over 10,000-fold.

Amphibian oogenesis is persistent in the adult ovary, with oogonia constantly differentiating into oocytes. This process is not synchronized; thus, individual lobes will contain oocytes in many stages of oogenesis. Interestingly, although this asynchronous and persistent development of oocytes from oogonia in adults was at one time considered specific only to lower vertebrates, evidence suggests that oogenesis may also be occurring persistently in the ovaries of female mice ***(12)***.

Generally, a cycle of oogenesis is considered complete when an ovary has a large population of banded stage VI oocytes, at which point the animal is ready to ovulate. Studies have shown that approx 5 to 7 wk are needed to repopulate the ovary with stage VI oocytes after human chorionic gonadotropin (hCG)-stimulated ovulation, suggesting that this amount of time is needed for oocytes to progress to ovulatory competence ***(13,14)***.

The criteria for staging *Xenopus* oogenesis are based on a scale developed by James Dumont ***(5)*** that allows identification of stages by morphologic appearance of unfixed oocytes. Given that oogenesis is a continuous process, no precise boundaries can be defined between stages; however, the features described here provide a basis for identifying the approximate stage of development and therefore predicting the physiologic processes occurring in a given oocyte. The salient features of each stage of oocyte development are reviewed in **Table 1** and are pictured in **Fig. 1**.

Table 1
Features of Oocytes at Stages I to VI of Development

	Stage					
	I	II	III	IV	V	VI
Size (μm)	50–300	300–450	450–600	600–1000	1000–1200	1200–1300
Features	Transparent	White	Pigmentation begins Light brown (early), dark brown-black (late)	Pigment polarizes: animal-vegetal hemispheres develop	Distinct hemispheres; pigment fades to brown	Formation of unpigmented equatorial band
Approximate percentage of stages II–VI population	NA	45%	15%	15%	10%	15%
Vitellogenesis[a]	—	—	+	+++	Decreasing	—

NA, not applicable.
[a]Vitellogenesis: stages I and II are considered previtellogenic; stage VI is considered postvitellogenic.

3.1. Stage I

Stage I oocytes are previtellogenic and range from 50 to 300 µm in diameter. A distinguishing feature of these oocytes is the transparent cytoplasm with an easily identified germinal vesicle that fills much of the cell. Large, irregular nucleoli are evident at the periphery of the nucleus by late stage I, and the nuclear envelope maintains a smooth outline. A densely packed collection of mitochondria that appears as a small, yellowish body, referred to as the mitochondrial mass or Balbiani body, can be visualized next to the nucleus. The cytoplasm usually appears diffusely granular, containing large amounts of RNA, ribosomes, and endoplasmic reticulum. In early stage I, follicle cells are tightly juxtaposed to the oocyte membrane; however, during late stage I, these follicle cells lift from the surface of the oocyte, and the microvilli begin to develop ***(15)***.

3.2. Stage II

Stage II oocytes are also previtellogenic and account for 45% of the total oocyte population in stages II to IV. Stage II oocytes range in diameter from 300 to 450 µm. At this point in development, oocytes acquire a characteristic opaque white color that obscures the mitochondrial mass and nucleus. Several changes begin to take place in the wall of the ovary and in the follicle cells during this stage:

1. Both the outer membrane and follicle cells grow in size, making these cell layers appear thicker.
2. Basement membranes develop on the basal (thecal) side of each cell layer.
3. The theca accumulates more collagen.
4. An expansive microvilli network that emanates from the apical side of the follicle cells and arches over the oocytes and the acellular vitelline envelope develops at the follicle cell-oocyte junction ***(1)***.
5. A periodic acid-Schiff (PAS)-positive granular layer develops along the periphery of the cytoplasm that contains cortical granules, mitochondria, small yolk platelets, lipid, and premelanosomes.
6. The nuclear envelope becomes progressively more irregular, and the nuclei acquire more irregularly shaped nucleoli along the periphery.

3.3. Stage III

Stage III oocytes range from 450 to 600 µm in diameter and account for 15% of the population in stages II to VI. Two important processes are initiated during stage III: acquisition of pigmentation and active uptake of yolk (vitellogenesis; *see* **Subheading 4.**). Pigmentation is initially evident by the tan or light brown appearance of oocytes, caused by melanin produced from the melanosomes lying beneath the cortical layer of the oocyte. During this stage, oocytes continue to darken uniformly, with no distinction between animal and vegetal poles, until the entire oocyte appears dark brown or black. Other morphologic changes during stage III include development of visible blood vessels along the surface of the oocyte, increased height of the follicle cells, increased numbers and sizes of microvilli, and continued development of the vitelline envelope ***(16)***.

Finally, nuclear changes also occur. First, formerly peripheral nucleoli become vacuolated and relocate to the center of the nucleus. Second, to allow for efficient transcription of genes to service the 10^5- to 10^6-fold higher volume of cytoplasm compared to somatic cells, the chromosomes relax into a lampbrush configuration. The presence of this conformation coincides with peak RNA synthesis, which begins in late stage II and lasts until very late stage III ***(17,18)***. In fact, stage III oocytes have their full complement of poly(A) mRNAs, but only a small fraction is translated ***(19)***.

3.4. Stage IV

Stage IV oocytes range in size from 600 to 1000 µm in diameter and account for approx 15% of the stage II to VI oocyte population. Differentiation of the animal and vegetal hemispheres becomes evident during stage IV. Determination of this axis is not dependent on where the follicle is attached to the ovarian wall. Instead, the axis of polarity seems to be established early in the oogonium based on a line that passes from the nucleus to the centrosome in these very early germ cells ***(20)***. Subsequent events, such as fragmentation of the mitochondrial mass during stage II development, accumulation of yolk platelets, and asymmetric thinning of the cortex, fall along this axis ***(21)***.

Other features typical of stage IV development are increased numbers of blood vessels in the theca and the continued growth of follicle cells. Channels begin to develop between adjacent follicle cells. Cortical granules become more uniform beneath the oocyte membrane, and the majority of melanosomes are now restricted to the animal pole. The cortex of the vegetal pole begins to thin, perhaps because of stretching, and the animal pole remains thick ***(22)***. The nuclear envelope remains convoluted, albeit less so than in the earlier stages, and the lampbrush chromosomes retract along with the nucleoli.

Vitellogenesis is most rapid during stage IV oogenesis. The yolk platelet density gradient from the animal to the vegetal axis becomes pronounced, and the nucleus seems to be displaced to the animal pole. The density gradient appears to develop because younger, smaller yolk platelets are imported via micropinocytosis at the animal pole and transported to the vegetal pole along intermediate filament-rich radii. During transit to the vegetal half of the oocyte, these small yolk platelets fuse to form older, larger, and more densely packed platelets ***(21)***.

3.5. Stage V

Stage V oocytes range in size from 1000 to 1200 µm and account for 10% of the oocyte population in stages II to VI. At this point in oogenesis, the border between the animal and vegetal hemispheres becomes more distinct, and the pigmentation of the animal hemisphere begins to fade from the dark brown of stage IV oocytes to a more brown or beige color. Blood vessels are still prominent, the vitelline envelope has reached maximal thickness, and follicle cells appear to flatten. At this point, vitellogenesis starts declining, although the yolk gradient continues to displace the nucleus toward the animal pole. At this point, the nuclear envelope polarizes such that the vegetal half of the nucleus becomes more irregular than the animal half, and the nucleoli and chromosomes condense to form a centrally located mass.

3.6. Stage VI

Stage VI oocytes mark the end of oocyte development and, because of the cessation of vitellogenesis, are considered postvitellogenic. As mentioned, by this stage oocytes have a banded appearance as a result of an unpigmented equatorial 200-µm band separating the heavily brown-pigmented animal pole from the pale yellow vegetal pole. The follicle cells continue to appear flattened, and the nuclei shrink in size. The vitelline envelope develops two distinct layers, and the number and length of microvilli extending from the oocyte surface are markedly reduced. A few nucleoli persist in the nucleus and tend to be localized near the highly convoluted vegetal half of the nuclear envelope ***(23)***, which is the side that breaks down first during initiation of meiosis (maturation).

At this stage, the oocyte has accumulated enough resources to support development of the embryo through the swimming tadpole stage. The inherent polarity that was established in the oogonia and that later became visually evident as the animal and vegetal poles during oocyte development is maintained during maturation and fertilization and ultimately becomes important for the appropriate formation of embryonic structures.

4. Vitellogenesis

Vitellogenesis refers to the formation of the oocyte yolk. *Xenopus* oocytes contain large quantities of yolk in the cytoplasm in the form of yolk platelets ***(7,24)***. In stage VI oocytes, these yolk platelets encompass nearly 80% of the total oocyte protein and appear as thousands of small cytoplasmic droplets when sectioned oocytes are viewed under the microscope. Yolk platelets are asymmetrically distributed in the cytoplasm. The animal hemisphere contains the lowest yolk content with the smallest yolk platelets, and the vegetal region contains the highest yolk content with the largest yolk platelets.

The yolk platelets consist of a combination of lipo-, glyco-, and phosphoproteins that are packed together in a crystalline fashion. They contain primarily two types of yolk: protein yolk, which contains phosphoproteins that are often attached to lipid molecules, comprises approx 45% of the oocyte's dry weight ***(25)***; fatty yolk, which consists of neutral lipids with varying amounts of phospholipids, makes up approx 25% of the oocyte mass. Most of the yolk content is synthesized outside the ovary and transported into oocytes during the secondary phase of oocyte growth.

For example, one of the major components of the yolk is the phosphoprotein vitellogenin, which appears in the bloodstream of female frogs during breeding season. Vitellogenin is synthesized in the liver, where its production is stimulated by estradiol but not other ovarian steroids such as androgens or progesterone ***(26)***. Although exogenous administration of estradiol increases vitellogenin production by the liver, vitellogenin uptake by the ooctyes remains low; thus, serum vitellogenin levels increase dramatically. In contrast, injection of exogenous gonadotropins into female frogs leads to liver vitellogenesis as well as rapid uptake of vitellogenin by the oocytes, resulting in relatively low serum vitellogenin levels.

The presumption is that gonadotropins stimulate vitellogenesis in the liver via ovarian estradiol production; vitellogenin uptake by oocytes is promoted through a separate, estradiol-independent, mechanism ***(27,28)***. Vitellogenin uptake begins when oocytes reach stage III of their growth and, although its mechanism is not completely understood, may involve pinocytotic and endocytotic events as well as communication between the follicle cells and oocytes via the gap junctions ***(29)***.

5. Steroidogenesis

Sex steroids appear to be secreted primarily by the ovaries of female frogs ***(30,31)***. During breeding season, serum estradiol levels increase, which stimulates vitellogenin production by the liver. The direct effects of estradiol on amphibian ovarian follicle development are still not well understood. Sex steroid production reaches a maximum during ovulation, when gonadotropins secreted from the pituitary stimulate both estrogen and androgen production; however, the exact amounts of the various ovarian steroids made in the ovaries during natural ovulation is not well documented. In contrast, both serum and ovarian steroid levels in female frogs injected with exogenous gonadotropins have been measured ***(32)***.

Injection of hCG promotes a rapid increase in ovarian sex steroid production that peaks after approx 8 h, which roughly coincides with ovulation. Exogenous hCG promotes moderate increases in estradiol production and dramatic increases in testosterone and androstenedione production. Although hCG stimulates ovarian progesterone production as well, its levels in the serum and ovaries relative to testosterone and estradiol remain quite low. This contrasts with gonadotropin-stimulated steroidogenesis in mammalian ovaries, where progesterone production exceeds that of androgens and estrogens.

Several studies have been directed toward characterizing the steroidogenic pathway in the *Xenopus* ovary. Together, these studies explain the high androgen, moderate estradiol, and low progesterone levels described. The classical ovarian steroidogenic pathway is depicted in **Fig. 2** ***(33)***.

After synthesis of the steroid pregnenolone from cholesterol, sex steroid production relies on four important enzymes. First, the cytochrome p450 enzyme CYP17 converts pregnenolone and progesterone to dehydroepiandrosterone (DHEA) and androstenedione, respectively. Second, the steroid dehydrogenase 3βHSD converts Δ5 steroids (such as pregnenolone and DHEA) to Δ4 steroids (progesterone and androstenedione, respectively). Third, 17β–hydroxysteroid dehydrogenase (17βHSD) metabolizes androstenedione to testosterone. Finally, the aromatase enzyme CYP19 converts androstenedione and testosterone to estrone and estradiol, respectively.

Several studies have demonstrated the presence of both 3βHSD and 17βHSD activities in *Xenopus* ovaries, localizing these enzymes primarily to follicle cells ***(34,35)***. In contrast, *Xenopus* CYP17 appears to be exclusively expressed in the oocytes themselves ***(32,35)***. Interestingly, pregnenolone is a very poor substrate for 3βHSD-mediated conversion to progesterone but is an excellent substrate for *Xenopus* CYP17. Thus, pregnenolone is preferentially converted to DHEA, which in turn is a good substrate for 3βHSD-mediated conversion to androstenedione. This selective

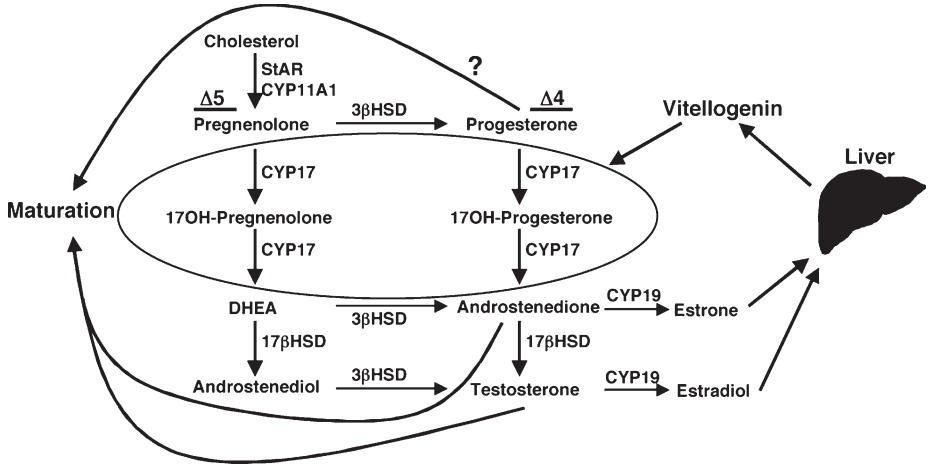

Fig. 2. Diagram of *Xenopus* ovarian steroidogenesis. CYP17 is expressed exclusively in the oocyte, which is represented by an oval shape in the center of the figure. All other steroidogenic enzymes are present in the surrounding follicle cells. Sex steroid production is therefore dependent on the germ cells, or oocytes. Estrogen and estrone enter the circulation to promote vitellogenesis in the liver. Vitellogenin then returns to the ovary and is taken up by oocytes. Androstenedione and testosterone are produced in large amounts prior to ovulation and may promote oocyte maturation. Although progesterone is also capable of promoting maturation, its production prior to ovulation is quite low; thus, the physiologic role of progesterone in regulating oocyte maturation is uncertain.

metabolism of pregnenolone via the Δ5 pathway likely explains why little progesterone is produced by the *Xenopus* ovary. Ovarian CYP19 activity, which seems to be primarily in follicle cells, is also relatively low, even in the presence of gonadotropins, thus explaining the reduced estradiol levels relative to testosterone ***(34)***.

The exclusivity of steroidogenic enzyme activities to specific cells within the ovary implies that *Xenopus* sex steroid production depends on both follicle cells and germ cells. This suggests an unusual positive-feedback model by which germ cells, or oocytes, are controlling their own growth and development by regulating synthesis of the steroids (estrogens) that promote vitellogenesis in the liver.

6. Oocyte Maturation and Ovulation

Just prior to ovulation, the microvilli between follicle cells and oocytes retract from the vitelline membrane so that the oocytes start to detach from the membrane ***(3)***. At the same time, chromosomal condensation begins, with spindle formation and loss of nuclear membrane definition (germinal vesicle breakdown). These features are part of oocyte maturation, which is defined as the resumption of meiosis beyond prophase I to metaphase II. Oocyte maturation is regulated by both pituitary and ovarian hormones.

In the 1930s, Rugh first demonstrated a role for pituitary factors in triggering amphibian ovulation by inducing the ovulation process with pituitary extracts ***(36)***. Shortly afterward, a role for ovarian factors in moderating ovulation was shown to be important through experiments in which ovulation in female frogs was triggered using ovarian tissue that had first been exposed to pituitary extract ***(37,38)***.

In the 1960s, progesterone was proposed to be the ovulation-inducing factor produced in the ovaries because submicromolar concentrations of progesterone promoted maturation in vitro ***(39)***. Since that time, nearly all of the seminal work studying meiosis in *Xenopus* oocytes has used progesterone as the trigger for maturation. However, many other steroids are equally or more capable of promoting oocyte maturation in vitro, including the androgens testosterone and androstenedione ***(32,39,40)***. Furthermore, as mentioned, gonadotropins stimulate very little ovarian progesterone production relative to these two androgens in vivo.

These observations suggest that androgens, rather than progesterone, may be the primary physiologic mediators of *Xenopus* oocytes in vivo. In addition, the CYP17 expressed in isolated oocytes rapidly converts progesterone to androstenedione; thus, in vitro "progesterone-induced maturation" likely involves androgen as well as progesterone actions ***(32)***. These observations suggest that, similar to the positive-feedback loop involving oocyte-dependent estrogen synthesis and oocyte growth via vitellogenesis, oocyte-mediated androgen production might also play a positive role in oocyte development by promoting maturation.

Investigation of the signaling mechanisms regulating steroid-induced oocyte maturation has been a subject of considerable interest for many decades ***(41)***. In contrast to most steroid-mediated signals, maturation appears to be transcription independent or nongenomic. Some nongenomic signals triggered by steroids during maturation include changes in intracellular cyclic adenosine monophosphate, promotion of the mitogen-activated protein kinase cascade, and activation of cyclin-dependent kinase 1. Many of these signals appear to involve steroid-induced changes in mRNA translation.

Interestingly, as mentioned, although transcription is very active during late oogenesis, only 20% of mRNA is translated ***(3)***. During maturation, a shift in the complement of translated mRNAs occurs, most likely because of changes in mRNA polyadenylation. One example is the MOS protein, a potent regulator of meiosis; its translation is increased by the addition of steroids ***(10,11,41)***. Studies suggest that these steroid-induced signals might in part involve classical steroid receptors that are signaling in a nongenomic fashion outside the nucleus. Other studies propose a "release of inhibition" model by which constitutive G protein-mediated signaling might be holding oocytes in meiotic arrest, and steroids might promote maturation by overcoming these inhibitory signals ***(42,43)***. More work is needed to confirm the validity of these models; however, studies in mammalian systems have shown that similar mechanisms may be present ***(44,45)***, emphasizing the usefulness of *Xenopus* oocytes as a general model for meiosis as well as for ovulation and oocyte development.

Acknowledgments

We thank Johné Liu for comments regarding this chapter. S. R. H. is funded by the National Institutes of Health (DK59913) and the Welch Foundation (I-1506). M. A. R. is funded in part by the Medical Scientist Training Program at the University of Texas Southwestern Medical Center.

References

1. Lofts, B. (1974) Reproduction, in *Physiology of the Amphibia* (Lofst, B., ed.), Academic Press, London, pp. 107–218.
2. Franchi, L. L. (1962) The structure of the ovary—vertebrates, in *The Ovary* (Zuckerman, S., ed.), Academic Press, New York, pp. 121–142.
3. Hausen, P. (1991) *The Early Development of* Xenopus laevis*: An Atlas of the Histology*, Springer-Verlag, Berlin.
4. Browne, C. L., Wiley, H. S., and Dumont, J. N. (1979) Oocyte-follicle cell gap junctions in *Xenopus laevis* and the effects of gonadotropin on their permeability. *Science* **203**, 182–183.
5. Dumont, J. N. (1972) Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* **136**, 153–179.
6. Scheer, U. (1973) Nuclear pore flow rate of ribosomal RNA and chain growth rate of its precursor during oogenesis of *Xenopus laevis*. *Dev. Biol.* **30**, 13–28.
7. Follett, B. K. and Redshaw, M. R. (1974) The physiology of the vitellogenesis, in *Physiology of the Amphibia* (Lofts, B., ed.), Academic Press, London, pp. 219–308.
8. Taylor, M. A. and Smith, L. D. (1985) Quantitative changes in protein synthesis during oogenesis in *Xenopus laevis*. *Dev. Biol.* **110**, 230–237.
9. Dolecki, G. J. and Smith, L. D. (1979) Poly(A)+ RNA metabolism during oogenesis in *Xenopus laevis. Dev. Biol.* **69**, 217–236.
10. de Moor, C. H. and Richter, J. D. (1997) The Mos pathway regulates cytoplasmic polyadenylation in *Xenopus* oocytes. *Mol. Cell Biol.* **17**, 6419–6426.
11. Mendez, R., Hake, L. E., Andresson, T., Littlepage, L. E., Ruderman, J. V., and Richter, J. D. (2000) Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. *Nature* **404**, 302–307.
12. Johnson, J., Canning, J., Kaneko, T., Pru, J. K., and Tilly, J. L. (2004) Germline stem cells and follicular renewal in the postnatal mammalian ovary. *Nature* **428**, 145–150.
13. Keem, K., Smith, L. D., Wallace, R. A., and Wolf, D. (1979) Growth rate of oocytes in laboratory maintained *Xenopus laevis*. *Gamete Res.* **2**, 125–135.
14. Smith, C. L. (1955) Reproduction in female amphibia. *Mem. Soc. Endocrinol.* **4**, 39–56.
15. Dumont, J. N. and Brummett, A. R. (1978) Oogenesis in *Xenopus laevis* (Daudin). V. Relationships between developing oocytes and their investing follicular tissues. *J. Morphol.* **155**, 73–98.
16. Kemp, N. E. (1958) Electron microscopy of growing oocytes of *Rana pipiens. J. Biophys. Biochem. Cytol.* **2**, 281–292.
17. Alfert, M. (1954) Comparison and structure of giant chromosomes. *Internal Rev. Cytol.* **3**, 131–175.
18. Hill, R. S. and Macgregor, H. C. (1980) The development of lampbrush chromosome-type transcription in the early diplotene oocytes of *Xenopus laevis*: an electron-microscope analysis. *J. Cell Sci.* **44**, 87–101.
19. Davidson, E. H. (1986) *Gene Activity in Early Development*, 3rd ed., Academic Press, New York.

20. Tourte, M., Mignotte, F., and Mounolou, J. C. (1981) Organization and replication activity of the mitochondrial mass of oogonia and previtellogenic oocytes in *Xenopus laevis*. *Dev. Growth Differ*. **23**, 9–21.
21. Danilchik, M. V. and Gerhart, J. C. (1987) Differentiation of the animal-vegetal axis in *Xenopus laevis* oocytes. I. Polarized intracellular translocation of platelets establishes the yolk gradient. *Dev. Biol.* **122**, 101–112.
22. Wylie, C. C., Brown, D., Godsave, S. F., Quarmby, J., and Heasman, J. (1985) The cytoskeleton of *Xenopus* oocytes and its role in development. *J. Embryol. Exp. Morphol.* **89 Suppl**, 1–15.
23. Coggins, L. W. (1973) An ultrastructural and radioautographic study of early oogenesis in the toad *Xenopus laevis*. *J. Cell Sci.* **12**, 71–93.
24. Follett, B. K., Nicholls, T. J., and Redshaw, M. R. (1968) The vitellogenic response in the South African clawed toad (*Xenopus laevis* Daudin). *J. Cell Physiol.* **72**, Suppl 1, 91+.
25. Barth, L. G. and Barth, L. J. (1951) The relation of adenosine triphosphate to yolk utilization in the frog's egg. *J. Exp. Zool.* **116**, 99–122.
26. Redshaw, M. R., Follett, B. K., and Nichollis, T. J. (1969) Comparative effects of the oestrogens and other steroid hormones on serum lipids and proteins in *Xenopus laevis* Daudin. *J. Endocrinol.* **43**, 47–53.
27. Wallace, R. A., Jared, D. W., and Nelson, B. L. (1970) Protein incorporation by isolated amphibian oocytes. I. Preliminary studies. *J. Exp. Zool.* **175**, 259–269.
28. Wallace, R. A., Nickol, J. M., Ho, T., and Jared, D. W. (1972) Studies on amphibian yolk. X. The relative roles of autosynthetic and heterosynthetic processes during yolk protein assembly by isolated oocytes. *Dev. Biol.* **29**, 255–272.
29. Wiley, H. S. and Dumont, J. N. (1978) Stimulation of vitellogenin uptake in stage IV *Xenopus* oocytes by treatment with chorionic gonadotropin in vitro. *Biol. Reprod.* **18**, 762–771.
30. Dodd, J. M. (1960) Gonadal and gonadotrophic hormones in lower vertebrates, in *Marshall's Physiology of Reproduction* (Parkes, A. S., ed.), Longmans Green, London, pp. 417–582.
31. Barr, W. A. (1968) Patterns of ovarian activity, in *Perspective in Endocrinology: Hormones in the Lives of Lower Vertebrates* (Jorgensen, E. J. W. Ba. C. B., ed.), Academic Press, New York, pp. 164–238.
32. Lutz, L. B., Cole, L. M., Gupta, M. K., Kwist, K. W., Auchus, R. J., and Hammes, S. R. (2001) Evidence that androgens are the primary steroids produced by *Xenopus laevis* ovaries and may signal through the classical androgen receptor to promote oocyte maturation. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 13,728–13,733.
33. Ozon, R. (1967) [In vitro synthesis of steroid hormones in the testicle and ovary of the urodele amphibian *Pleurodeles waltlii* Michah]. *Gen. Comp. Endocrinol.* **8**, 214–227.
34. Redshaw, M. R. and Nicholls, T. J. (1971) Oestrogen biosynthesis by ovarian tissue of the South African clawed toad, *Xenopus laevis* Daudin. *Gen. Comp. Endocrinol.* **16**, 85–96.
35. Yang, W. H., Lutz, L. B., and Hammes, S. R. (2003) *Xenopus laevis* ovarian CYP17 is a highly potent enzyme expressed exclusively in oocytes. Evidence that oocytes play a critical role in *Xenopus* ovarian androgen production. *J. Biol. Chem.* **278**, 9552–9559.
36. Rugh, R. (1935) Ovulation in the frog. I. Pituitary relations in induced ovulation. *J. Exp. Zool*. **71**, 149–162.
37. Heilbrunn, L. V., Daugherty, K., and Wilbur, K. M. (1939) Initiation of maturation in the frog egg. *Physiol. Zool*. **12**, 97–100.
38. Ryan, F. J. and Grant, R. (1940) The stimulus for maturation and for ovulation of the frog's egg. *Physiol. Zool*. **13**, 383–390.

39. Smith, L. D., Ecker, R. E., and Subtelny, S. (1968) In vitro induction of physiological maturation in *Rana pipiens* oocytes removed from their ovarian follicles. *Dev. Biol.* **17**, 627–643.
40. Le Goascogne, C., Sananes, N., Gouezou, M., and Baulieu, E. E. (1985) Testosterone-induced meiotic maturation of *Xenopus laevis* oocytes: evidence for an early effect in the synergistic action of insulin. *Dev. Biol.* **109**, 9–14.
41. Maller, J. L. and Krebs, E. G. (1980) Regulation of oocyte maturation. *Curr. Top. Cell Regul.* **16**, 271–311.
42. Lutz, L. B., Kim, B., Jahani, D., and Hammes, S. R. (2000) G protein βγ subunits inhibit nongenomic progesterone-induced signaling and maturation in *Xenopus laevis* oocytes. Evidence for a release of inhibition mechanism for cell cycle progression. *J. Biol. Chem.* **275**, 41,512–41,520.
43. Sheng, Y., Tiberi, M., Booth, R. A., Ma, C., and Liu, X. J. (2001) Regulation of *Xenopus* oocyte meiosis arrest by G protein βγ subunits. *Curr. Biol.* **11**, 405–416.
44. Gill, A., Jamnongjit, M., and Hammes, S. R. (2004) Androgens promote maturation and signaling in mouse oocytes independent of transcription: a release of inhibition model for mammalian oocyte meiosis. *Mol. Endocrinol.* **18**, 97–104.
45. Conti, M., Andersen, C. B., Richard, F., et al. (2002) Role of cyclic nucleotide signaling in oocyte maturation. *Mol. Cell Endocrinol.* **187**, 153–139.