Zygote **7** (February), pp 11–19. © 1999 Cambridge University Press Printed in the United Kingdom

Comparative study of vitellogenesis in the anuran amphibians *Ceratophrys cranwelli* (Leptodactilidae) and *Bufo arenarum* (Bufonidae)

Evelina I. Villecco, Manuel J. Aybar, Alicia N. Sánchez Riera and Sara S. Sánchez
Departamento de Biología del Desarrollo, Instituto Superior de Investigaciones Biológicas (INSIBIO), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) y Universidad Nacional de Tucumán (UNT), Chacabuco 461, 4000-San Miguel de Tucumán, Tucumán, Argentina

Date submitted: 23.5.98. Date accepted: 2.7.98

Summary

The present study analyses, by transmission electron microscopy, vitellogenesis in two anuran amphibian families: Leptodactilidae (*Ceratophrys cranwelli*) and Bufonidae (*Bufo arenarum*). These differ in the type of stimulus that sets off their reproductive period, pluvial changes being the trigger in *C. cranwelli* and temperature increase in *B. arenarum*. We found that vitellogenesis follows an endocytic pathway that involves membranous structures (coated pits, coated vesicles, endosomes and multivesicular bodies). This process results in a fully grown yolk platelet of similar structure in both species. Despite the above similarity, a distinctive feature in *B. arenarum* was that the multivesicular bodies exhibited condensed proteins together with lipid droplets, the latter remaining as such even in the primordial yolk platelet. In *C. cranwelli*, however, lipids droplets were only found attached to the primordial yolk platelet. The coexistence of lipid droplets together with proteins in the nascent precursor yolk platelets observed in *B. arenarum* is similar to that found in *B. marinus*. This fact might constitute a characteristic feature of the Bufonidae family.

Keywords: Amphibian, Cytoplasmic organelles, Oocyte, Vitellogenesis, Yolk platelet

Introduction

In most oviparous animals, oogenesis is characterised by an abundant accumulation of yolk proteins that provide the nutritive materials needed for embryogenesis (Karasaki, 1963; Wallace, 1985; Komasaki, 1987).

Vitellogenin (VTG), the macromolecular precursor of the yolk proteins, is normally a female-specific protein selectively sequestered by growing oocytes. Much of the work on VTG in amphibian (*Xenopus laevis*) oocytes has demonstrated that VTG is incorporated by the oocyte and proteolytically cleaved into yolk proteins: lipovitellin and phosvitin (Wiley *et al*., 1981; Opresko & Wiley, 1987*a*–*c*).

The injection of oestradiol-17β into amphibian females enhances the synthesis of the yolk precursor in the liver (Wallace *et al*., 1968; Shapiro, 1982; Varriale *et al*., 1988). VTG is then transported via the blood stream to the ovary, where it successively passes the endothelium, the basal lamina, the follicular epithelium and the vitelline envelope until it reaches the oocyte, where it is recognised by specific plasma membrane receptors and selectively internalised by receptor-mediated endocytosis (Opresko & Wiley, 1987*a*, *b*; Stifani *et al*., 1988; 1990*a*). The internalised products are then stored in a stable form, the yolk, until embryogenesis occurs (Karasaki, 1963; Komasaki *et al*., 1987).

In amphibians, VTG is by far the most important yolk precursor protein. Vitellogenins are phosphoglycoproteins composed of two subunits of molecular weight that range from 180 000 to 240 000 according to species (Stifani *et al*., 1990*b*). They seem to have been conserved throughout evolution since, when sequence information is available, as in the case of birds (chickens), amphibians (*Xenopus laevis*) and fish (salmon), it is evident that vitellogenins from different species are closely related not only functionally but also structurally.

All correspondence to: S.S. Sánchez, Departamento de Biología del Desarrollo, INSIBIO (CONICET-UNT), Chacabuco 461, 4000-San Miguel de Tucumán, Argentina. Fax: +54–81–248025. e-mail: mjaybar@unt.edu.ar.

The specific transport pathway of VTG in amphibian oocytes has been investigated by several authors (Wall & Meleka, 1985; Wall & Patel, 1987*a*; Richter, 1989; etc.). In amphibians, the *Xenopus* oocyte is one of the main experimental systems for the study of the events following internalisation of VTG. Transport of VTG proceeds from oolemma via coated vesicles and multivesicular endosomes to primordial yolk platelets (types I and II) and finally to fully grown yolk platelets. The pathway of incorporation of lipids into the vitelline platelet, however, has received little attention. The platelets are reservoirs for a variety of biomolecules such as amino acids and proteins (Wallace, 1970), lipids and carbohydrates (Robertson, 1979) and sequestered alkali ions (Ibrahim, 1980; Lynn, 1984). The incorporation of reserve substances into the vitelline platelets has not been completely elucidated; taking into account the existence of different families, genera and species with various reproductive patterns among amphibians, it seems that the process of formation of the vitelline platelet would also present differences in each case.

When considering vitellogenesis in the better-known amphibians (*Xenopus laevis, Rana pipiens*), it may be that each species has common as well as unique features that might be particularly useful for analysing different aspects of this process. This investigation focuses on the pathway of the deposition of the yolk proteins and lipids in the oocyte of two species of anurans with different ovarian cycles and modes of reproduction: *Ceratophrys cranwelli* (Leptodactilydae) and *Bufo arenarum* (Bufonidae). The oogenesis of *C. cranwelli*, distributed in the northeast of Argentina, was studied. This aggressive species is a voracious flesh-eater, feeding on small vertebrates and arthropods. During the dry season it remains underground inside a queratinous cocoon in a latent condition for in excess of 2 years, until a summer flood occurs, at which time it becomes active at once. It is then that the coupling and reproductive periods start. Even though no studies exist regarding the above, this particular behaviour might be due to the need of ensuring reproduction within an extremely limited time. Its seasonal frequency occurs at random, in accordance with the yearly rainfalls. The other species studied was *Bufo arenarum* which, in contrast to *C. cranwelli*, has a yearly reproductive cycle. Its ovulation occurs regularly once a year, during the spring season, and the follicles and oocytes grow and differentiate as a single cohort during the summer months. Thus, by the time the animals enter natural hibernation, large populations of uniformly sized hormone-responsive follicles exist in arrested meiosis within the ovary.

Materials and methods

Animals

Adult specimens of *Bufo arenarum* and *Ceratophrys cranwelli* were collected in the northeast area of Argentina from September to December (breeding season) and kept at 15 °C until use, which generally took place 15 days after collection. Sometimes the animals were used immediately.

Chemicals

Oestradiol-17β (1,3,5,[10]estratriene-2,4,-3,17-β-diol) 1 mg/ml and progesterone (4-pregnen,3,10,dione) (Sigma) were dissolved in drops of absolute alcohol, and the solutions brought up to volume by addition of Ringer solution. Human chorionic gonadotrophin (hCG: Sigma) was prepared as stock solution at 1 mg/ml concentration in Ringer solution.

Tissue preparation for microscopy

Sexually mature females were anaesthetised. After laparoscopy, mesovari were carefully dissected to obtain isolated oocytes at stages III–VI (Dumont, 1972) wrapped in their theca follicle. Isolated follicles were measured with an eyepiece micrometer in a microscope stereoscope.

Transmission electron microscopy

For thin sectioning, entire follicles were fixed for 4 h at 4 °C in 2.5% glutaraldehyde in 0.1% M sodium phosphate (pH 7.4). Afterwards follicles were washed twice in phosphate buffer and post-fixed in 1% osmium tetroxide in the same buffer at 4 °C overnight. Samples were dehydrated in an ethanol series and embedded in Spurr resin. Sectioning was carried out with Potter Blum MT1 ultramicrotome. Slices were stained with lead citrate and uranyl acetate. Preparations were examined with a Zeiss EM electron microscope.

Freeze-fracture

For freeze-fracture platinum replicas, the follicles were removed from the ovary and fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 24 h at 4 °C. They were then immersed for an additional 30 min in the same fixative and, after brief washing in buffer, placed in 25% glycerol in distilled water for between 2 h and 2 days. The follicles were mounted on gold supports and then rapidly frozen in liquid Freon 22 and subsequently stored in liquid nitrogen. Freeze-fracturing and replicas were made in a Balzers B.A. 301 at –105 °C. No etching was carried out. The carbon

https://doi.org/10.1017/S0967199499000349 Published online by Cambridge University Press

platinum replicas were cleansed with sodium hypochlorite and mounted on uncoated copper grids.

Horseradish peroxidase labelling

Adult females of *Ceratophrys cranwelli* were stimulated by injection of 4 mg of oestadiol-17β per 100 g body weight (Wallace *et al*., 1980) in order to enhance synthesis of VTG in the liver. Five days later the animals were injected in the dorsal lymphatic sac with 20 mg/100 g body weight horseradish peroxidase (HRP; MW 40 000; Type II, Sigma) dissolved in 1 ml amphibian Ringer solution and 400 IU hCG to stimulate VTG and HRP incorporation by the oocytes. The animals were anaesthetised in order to remove a piece of the ovary at 1 h and 24 h after injection of HRP. Vitellogenic measured follicles were fixed in 2.5% glutaraldehyde in sodium phosphate buffer (pH 7.4) for 4 h. After washing in the same buffer for 2–4 h experimental follicles were rinsed in 0.05 M phosphate buffer (pH 7.4) and incubated in 0.05% diaminobenzidine (DAB; Sigma) containing 0.01% hydrogen peroxide in phosphate-buffered saline (pH 7.4) for 20–40 min (Graham & Karnovsky, 1966) and 1 mg aminotriazole. The aminotriazole was included to inhibit the endogenous catalase activity.

As a control experiment one animal received an injection of 1 ml Ringer solution without the addition of HRP, followed by incubation as described for the experiments. Additional control follicles from injected frogs were also incubated in DAB reaction mixture but hydrogen peroxide was omitted from the medium. After several buffer washes, follicles were post-fixed in osmium tetroxide and processed for electron microscopy as previously outlined.

Results

The oogenesis of *Ceratophrys cranwelli* and *Bufo arenarum* were investigated by transmission electron microscopy. The ovaries of a *C. cranwelli* adult female consist of about 13 lobes in two zones: (a) proximal zone containing oocytes types I, II, III (previtellogenic and early vitellogenic) and (b) a distal zone containing oocytes in stages IV, V, VI (Villecco, 1998). The ovaries of *B. arenarum* examined in spring contained more than 25 lobes and mainly fully grown oocytes (about 1.7–1.8 mm diameter), vitellogenic oocytes (250–1200 µm) and a few transparent oocytes (45–250 µm) (Valdez Toledo, 1978).

In both *C. cranwelli* and *B. arenarum* stage III of oogenesis represents the beginning of the vitellogenic process characterised by the incorporation and accumulation of yolk. Examination of *C. cranwelli* oocytes (stages IV and V) by transmission electron microscopy revealed a folded surface. This surface contained numerous coated pits and the cortical ooplasm presented coated vesicles (Fig. 1*A*). The fusion of coated vesicles with each other, or with cortical endosomes, was observed. After fusion the small vesicles, clathrine-coated, became completely incorporated and inserted into the growing endosome, transforming it into a multivesicular endosome (Fig. 1*B*). this process was also seen in stimulated females of *B. arenarum*; however, in routinely prepared specimens of unstimulated naturally maintained females, the endocytotic activity was low. In the multivesicular endosome of *C. cranwelli*, VTG condensation and crystallisation occurred, forming electrodense masses; these gradually grew into multivesicular bodies that represented the initial yolk platelet precursor (Fig. 1*B*). These structures developed into two nascent yolk organelles (primordial yolk platelet types I and II). The type I yolk precursors are small and stratified in whorls and their proteolipids, which were packed in a random paracrystalline lattice at different angles to each other, were perforated by yolk-free tubular cavities. Primordial yolk platelet type II usually developed in the subcortical cytoplasm. Its origin was the small precursor assembly by vesicle fusion. It contained only one crystalline core and the proteolipid sheets were uniformly stratified. This precursor was frequently attached to another yolk platelet (Fig. 1*C*). The growing yolk platelet was accompanied by lipid droplets, which were in intimate contact with the boundary of the platelets until the droplets became incorporated across the membrane (Fig. 1*D*).

In contrast to *C. cranwelli*, in *B. arenarum* lipid droplets occupied a considerable volume of the ooplasm simultaneously with the formation of multivesicular endosomes (Fig. 2). after the lipids had been enclosed in the endosome, they formed a multivesicular body with evident lipid droplets, where lipoproteins condensed to form a paracrystalline core, either in the centre or near the periphery. These nascent yolk precursors could become fused with each other to form a membrane-limited yolk platelet, which always enclosed lipid droplets. Each of the internalised cores initially retained its paracrystalline lattice but eventually the lipoprotein material was recrystallised.

As is typical for other species the vitellogenic organelles of *C. cranwelli* and *B. arenarum* developed into characteristic fully grown yolk platelets. They contained one single main body where the lipoproteins were packed in a crystalline lattice in sheets oriented at the same angle and were deprived of the lipid droplets. In freeze-fracture images, the crystal core of *C. cranwelli* and *B. arenarum* primordial precursor yolk platelets (Fig. 3) showed a lipovitellin-complex stratification, but abundant lipid droplets surrounding the crystal core were observed only in *B. arenarum* oocytes (Fig. 3*B*).

https://doi.org/10.1017/S0967199499000349 Published online by Cambridge University Press

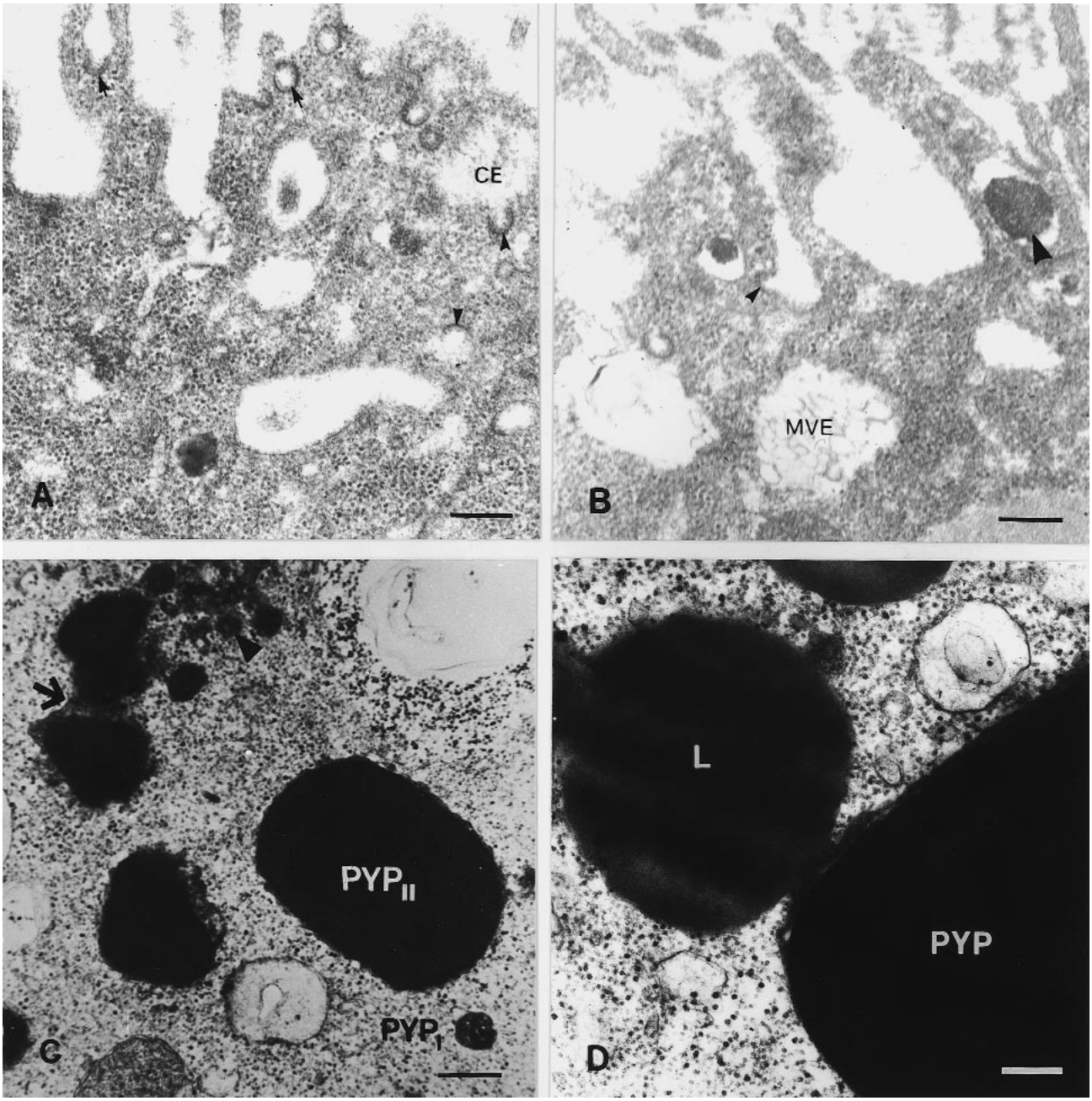

Figure 1 Electron micrographs of *Ceratrophrys cranwelli* vitellogenic follicles. (*A*) Oocyte cortex showing a folded surface. Arrows, coated pits and coated vesicles; arrowheads, fusion of coated vesicles with cortical endosomes; CE, cortical endosome. Scale bar represents 0.29 µm. (*B*) Oocyte cortex showing a multivesicular endosome and a multivesicular body. Small arrowhead, fusion of coated vesicles with cortical endosome; large arrowhead, multivesicular body; MVE, multivesicular endosome. Scale bar represents 0.3 µm. (*C*) Oocyte cortex showing yolk platelet precursors. Large arrowhead, multivesicular body; arrow, fusion of type II primordial yolk platelet; PYP_I, type I primordial yolk platelet; PYP_{II}, type II primordial yolk platelet. Scale bar represents 0.39 µm. (*D*) Oocyte cortex showing fusion of a lipid droplet with a primordial yolk platelet. PYP, type II primordial yolk platelet; L, lipid droplet. Scale bar represents 0.29 µm.

To confirm the pathway of maternal protein transport during the vitellogenesis of *C. cranwelli*, we used the macromolecular tracer horseradish peroxidase (HRP). This was chosen since it can readily be localised at the ultrastructural level. HRP was injected into female *C. cranwelli* that had previously been treated with oestradiol-17β to induce vitellogenesis and then with hCG to stimulate the uptake of proteins as stated above (Materials and Methods). One hour after HRP injection the reaction products were found within the

https://doi.org/10.1017/S0967199499000349 Published online by Cambridge University Press

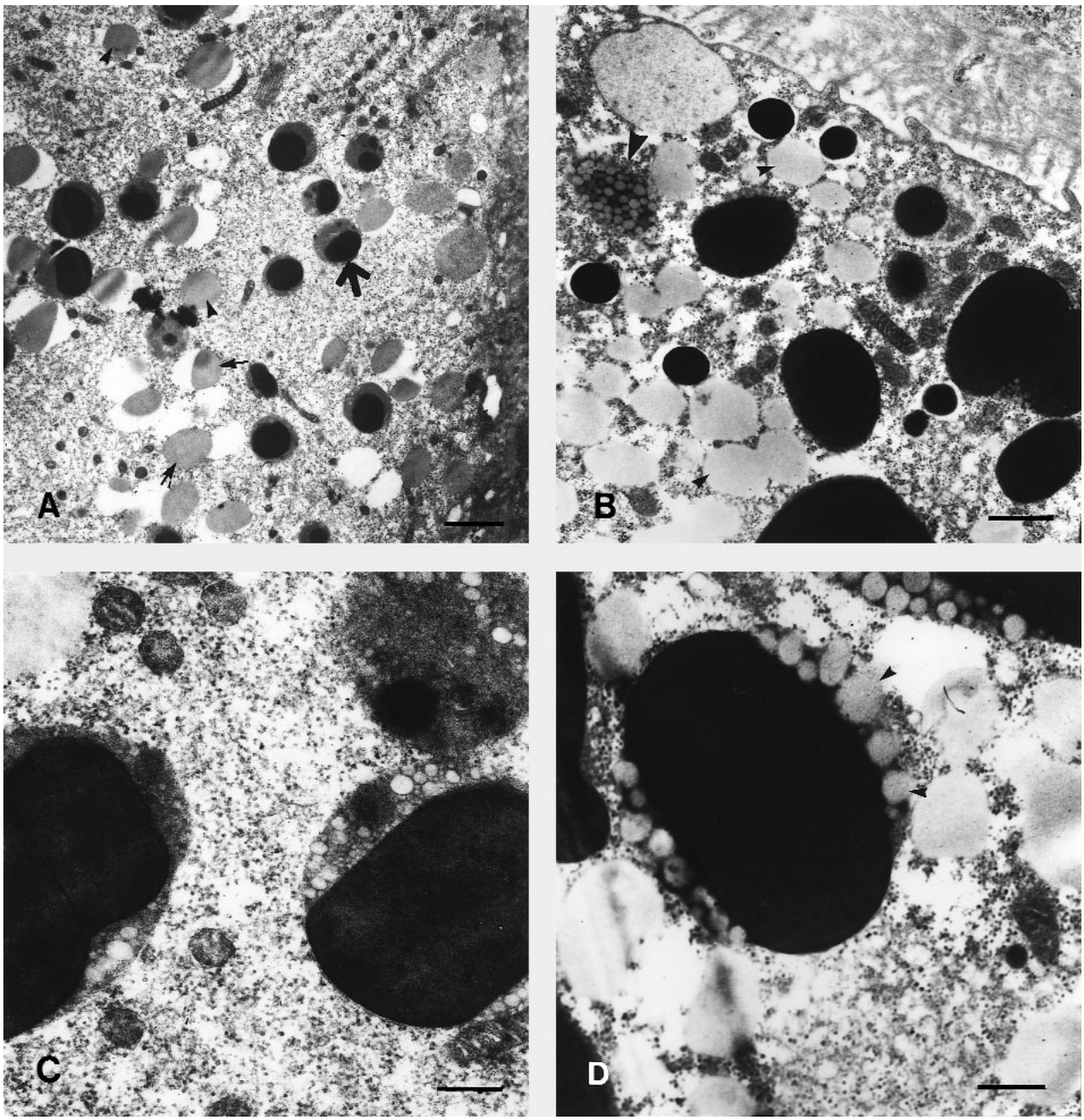

Figure 2 Electron micrographs of *Bufo arenarum* vitellogenic follicles. (*A*) Oocyte cortex showing abundant lipid droplets in close proximity to endosomes and multivesicular bodies. Arrowheads, lipid droplets; small arrows, lipid droplets in endosomes; large arrow, multivesicular body. Scale bar represents 1.4 μm. (*B*) Oocyte cortex of an unstimulated female showing numerous lipid droplets and yolk platelet precursors. Large arrowhead, multivesicular body; small arrowheads, lipid droplets. Scale bar represents 0.5 μm. (*C*) Multivesicular bodies in different degrees of yolk condensation. Scale bar represents 1.40 μm. (*D*) Primordial yolk platelet with lipid droplets. Arrowheads, lipid droplets. Scale bar represents 0.29 μm.

capillaries and within the intercellular space between adjacent endothelial cells. Fig. 4*A* depicts regions of vitellogenic follicles in which HRP reaction products were seen distributed among the fibroblast and collagen fibres of the theca. Macromolecular materials thus appear to pass the basal lamina and the overlying follicular epithelium, being found within the intercellular spaces between the follicular cells as well as within the pores of the vitelline envelope (Fig. 4*B*, *C*).

Maternal proteins have a unidirectional transport pathway. HRP reaction products were visible within coated pits and endocytic vesicles together with smooth-surface tubules found in the cortical ooplasm (Fig. 4*D*). Several electrodense yolk spheres containing reaction

https://doi.org/10.1017/S0967199499000349 Published online by Cambridge University Press

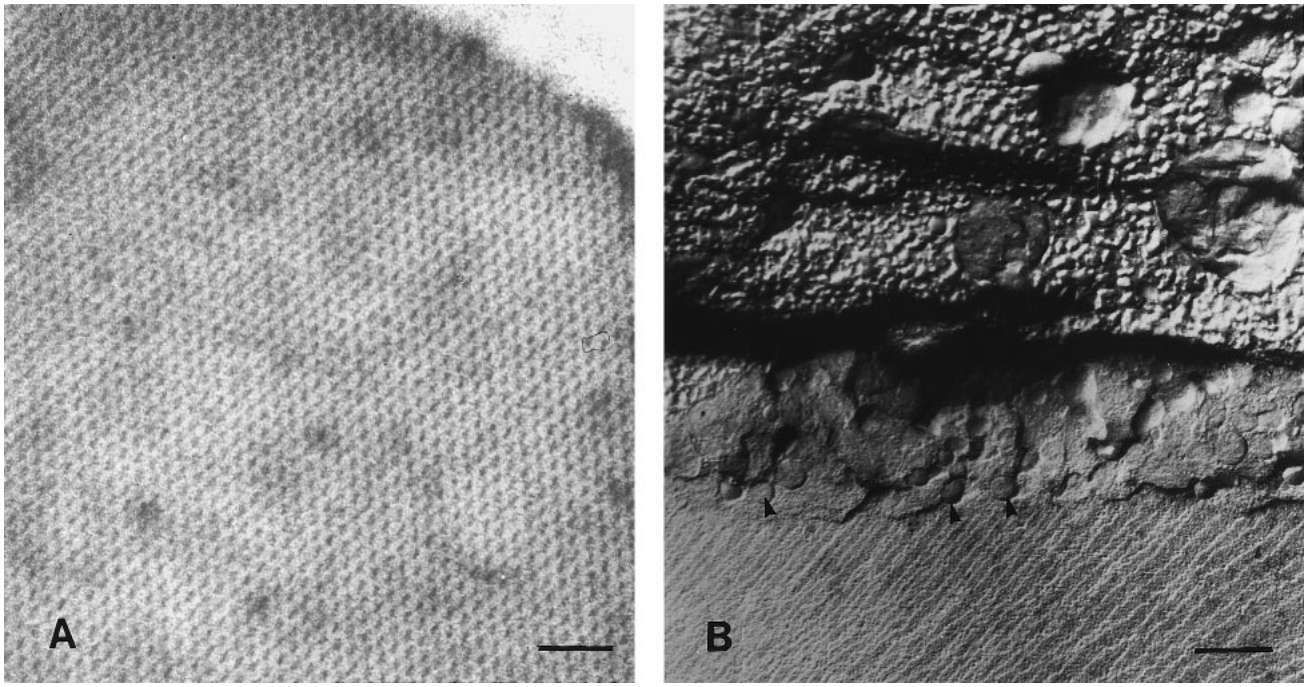

Figure 3 Structure of fully grown yolk platelet and primordial yolk platelet. (*A*) Thin section of the crystalline lattice of *Ceratophrys cranwelli* and *Bufo arenarum* fully grown yolk platelet. Scale bar represents 0.042 µm. (*B*) Freeze-fracture replica of a *Bufo arenarum* primordial yolk platelet, showing the absorption of lipid droplets (arrowheads) into yolk precursor. Scale bar represents 1.0 µm.

products were observed. Twenty-four hours after HRP injection, the reaction products were observed in the fully grown yolk platelets. Control follicles from injected females incubated in the absence of hydrogen peroxide revealed no reaction products at any site (Fig. 4*A*, insert). The same results were obtained in *B. arenarum* (data not shown).

Discussion

In this study the vitellogenesis of two species of different anuran families, Leptodactylidae and Bufonidae, was investigated. The transport of VTG inside the ovary of *Ceratophrys cranwelli* and *Bufo arenarum*, as in the case of amphibians such as urodeles (newts, salamanders) and anurans (*Xenopus laevis, Rana pipiens, Bufo marinus*, etc.), proceeds from blood capillaries across the basal lamina and follicle cells until it reaches the oocyte.

The study of the endocytic process is very attractive because of its unidirectional pathway that transports receptor-mediated VTG from coated pits directly to oocyte depots. Fully grown yolk platelets are the destination of most of the proteinaceous nutritive material to be internalised. Under our conditions, we demonstrated that the intact functional membrane compartments were involved in the internalisation of the VTG and other proteins (vitronectin: Aybar *et al*., 1996; cathepsin: Yoshizaki & Yonezawa, 1994). With HRP we were able to follow the pathway from the oocyte surface to the yolk organelle in more detail. In both *C. cranwelli* and *B. arenarum* the hormonal stimulation by oestradiol-17β and hCG produced a highly tortuous surface that greatly increased the endocytic membrane area of the oocyte.

On the basis of our results for *C. cranwelli* we may postulate the following pathway for VTG and other proteins such as peroxidase: VTG was internalised into the oocyte via coated pits and coated vesicles. The coated vesicles fused with each other and with cortical tubular endosomes. These tubular membrane compartments probably represent the definitive site of an uncoupling of receptor and ligand forming nascent multivesicular endosomes. Yolk finally condensed inside endosomes to develop a typical multivesicular body, which grew and formed primordial platelets. Based on diverse experimental data obtained in *Xenopus laevis*, Wallace & Selman (1990) proposed a fundamental role for the multivesicular body in VTG processing. Dissociation of VTG and its receptor would occur in this organelle (Wall & Patel, 1987*a*). Passage of VTG via multivesicular bodies seems to be required for cleavage and platelet formation (Wall & Patel, 1987*b*). At the ultrastructural level, however, it was not possible to visualise the presence of lysosomal activity in the multivesicular bodies; consequently, it could only be presumed that these bodies were potential contribu-

https://doi.org/10.1017/S0967199499000349 Published online by Cambridge University Press

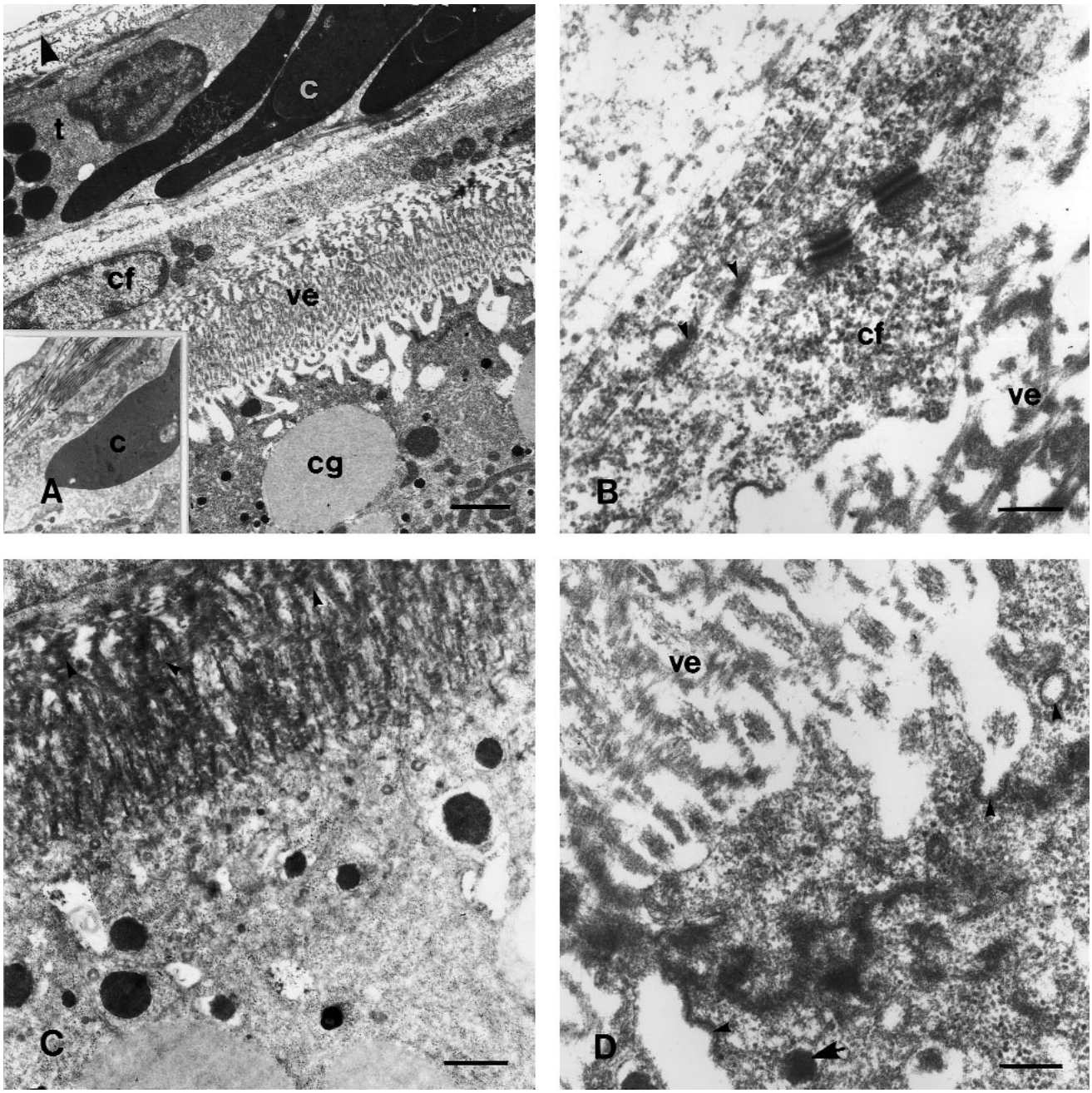

Figure 4 Electron micrographs of the outer region of *Ceratophrys cranwelli* vitellogenic follicles from HRP-injected animals. (*A*) After 1 h, HRP injection products were located within the vascular components of the theca. Insert: control follicle showing no reaction products. C, capillaries. Scale bar represents 2 µm. (*B*) HRP reaction products after 2 h of injection are located within the intercellular space between follicle cells (arrowheads). Scale bar represents 0.29 µm. (*C*) HRP reactions products (arrowheads) visible in vitelline envelope tunnels 3 h after injection. Scale bar represents 0.80 µm. (*D*) HRP reaction products are located within coated pits, endocytic vesicles and smooth-surfaced tubules of the oocyte cortex 4 h after injection. Scale bar represents 0.29 µm.

tors to hydrolase activity and that they were the site of partial proteolysis of VTG (Sire *et al*., 1994).

The results in *Bufo arenarum* demonstrated that the VTG was incorporated following a pattern similar to that of *Ceratophrys cranwelli* and *Xenopus laevis* (Richter, 1989, 1990) in contrast to *Bufo marinus* in which no clear evidence for this kind of protein transport has been found (Richter, 1987).

Another relevant aspect to be considered in the study of vitellogenesis is the incorporation of lipids into yolk platelets. Materials responsible for oocyte development, mainly lipids, are synthesised in the

https://doi.org/10.1017/S0967199499000349 Published online by Cambridge University Press

liver and released into the circulation, and could be incorporated by fatty bodies and transferred to the ovary to be incorporated into growing yolk platelets (Milone *et al*., 1978).

Our transmission electron microscopy results showed a different pattern for lipid incorporation in the precursor yolk organelles. In *C. cranwelli* lipid droplets are tightly attached to the membrane of primordial yolk platelets and were probably incorporated inside the organelle. In contrast, some differences in *B. arenarum* were found. The incorporation of lipid material into the membranous structure has not been thoroughly elucidated. It is possible that the lipids directly penetrate the membrane and remain as small lipid droplets inside the organelle.

Electron microscopic findings of *B. arenarum* yolk precursors are similar to those observed in *B. marinus* and differ from those in *C. cranwelli* and *X. laevis*. In these latter two distinctly related species (Duellman & Trueb, 1986) lipid droplets inside the yolk organelles are not evident.

The precursor of yolk organelles can be considered as a 'fluid crystal', probably due to the high content of initially unbound lipids and the 15–30% water in the crystalline core (Wallace, 1963). In addition, the large amount of internalised lipids should cause an increased fluidity of the lipid phase, thus providing enough space between the different paracrystalline initial core to allow movement of lipoprotein molecules that would finally result in one solid crystal with one direction of molecular packaging in the fully grown yolk platelet (Lange, 1985).

In brief, the imaging findings in vitellogenic oocytes of *Ceratophrys cranwelli* are similar to those of *Xenopus laevis*, even though these species belong to different families. This might be due to the fact that both species are capable of carrying out an accelerated vitellogenesis stimulated by pluvial changes – a factor that seems to be responsible for the activation of the reproductive process (Tinsley *et al*., 1996). This would represent a contrast with *Bufo arenarum* and *Bufo marinus*, both belonging to the Bufonidae family, in which climatic changes, mainly temperature, start the above process. The presence of lipid droplets together with proteins in the multivesicular bodies observed in the species of the Bufonidae family might constitute a characteristic feature in this family that could be related to the reproductive process; however, further investigations are required on the subject.

References

Aybar, M.J., Genta, S.B., Villecco, E.I., Sánchez Riera, A.N. & Sánchez, S.S. (1996). Amphibian *Bufo arenarum* vitronectin-like protein: its localization during oogenesis. *Int. J. Dev. Biol.* **40**, 9977–1008.

Duellman, W.E. & Trueb, L. (1986). *Biology of Amphibian*. New York: McGraw-Hill.

Dumont, J.N. (1972). Oogenesis in *Xenopus* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* **136**, 153–80.

Graham, R.C. & Karnovsky, M.J. (1966). The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**, 291–302.

Ibrahim, K.B. (1980). Estimation of sodium and potassium in platelets of toad (*Bufo bufo*) oocytes by electron microprobe, X-ray analysis. *J. Physiol. (Lond.)* **305**, 91–2.

Karasaki, S. (1963). Studies on amphibian yolk. 5. Electron microscopic observation on the utilization of yolk platelets during embryogenesis. *J. Ultrastruct. Res.* **9**, 225–47.

Komasaki, S. (1987). A yolk-granule component acts as an adhesive material for dissociated gastrula cells of the newt. *Cynops pyrroghaster*. *Dev. Growth Differ*. **29**, 517–26.

Komazaki, S. & Asashirma, M. (1987). Structural changes of yolk platelets and related organelles during development of the newt embryo. *Dev. Growth Differ.* **29**, 323–31.

Lange, R. (1985). The vertebrate yolk platelet crystal: comparative analysis of an *in vitro* crystalline aggregate. *Int. Rev. Cytol.* **97**, 133–81.

Lyn, G.N. (19840. Counter arguments against alleged proof of the Na–K pump in studies of K^+ and Na^+ distribution in amphibian eggs. *Physiol. Chem.* **16**, 293–305.

Milone, M., Iela, L., Esposito, V., Rastogi, R.K. & Chieffi, G. (1978). Annual variation in the total lipid and protein content of the liver, fat body, ovary and plasma of female frog (*Rana esculenta* L.). *J. Endocrinol.* **78**, 165–9.

Opresko, L.K. & Wiley, H.S. (1987*a*). Receptor-mediated endocytosis in *Xenopus* oocytes. I. Characterization of the vitellogenin receptor system. *J. Biol. Chem.* **261**, 4109–15.

Opresko, L.K. & Wiley, H.S. (1987*b*). Receptor mediated endocytosis in *Xenopus* oocytes. II. Evidence for two novel mechanism of hormonal regulation. *J. Biol. Chem.* **262**, 4116–23.

Opresko, L.K. & Wiley, H.S. (1987*c*). Specific proteolysis regulates fusion between endocytic compartments in *Xenopus* oocytes. *Cell* **51**, 557–68.

Richter, H.P. (1987). Membranes during yolk-platelet development in oocytes of the toad *Bufo marinus*. *Rouxs Arch. Dev. Biol.* **196**, 367–71.

Richter, H.P. (1989). Yolk organelles and their membranes during vitellogenesis in *Xenopus* oocytes. *Rouxs Arch. Dev. Biol.* **198**, 92–102.

Richter, H.P. & Bower, A. (1990). Ferromagnetic isolation of endosomes involved in vitellogenin transfer into *Xenopus* oocytes. *Eur. J. Cell Biol.* **51**, 53–63.

Richter, H.P., Jung, D. & Passow, H. (1984). Regulatory changes of membrane transported ouabain binding during progesterone-induced maturation of *Xenopus* oocytes. *J. Membr. Biol.* **79**, 203–10.

Robertson, N. (1979). Carbohydrate content of isolated yolk platelets from early developmental stages of *Xenopus laevis*. *Cell Differ*. **81**, 173–85.

Shapiro, D. (1982). Steroid hormone regulation of vitellogenin gene expression. *Rev. Biochem.* **12**, 187–203.

Sire, M.F., Babin, P.J. & Vernier, J.M. 91994). Involvement of

the lysosomal system in yolk protein deposit and degradation during vitellogenesis and embryonic development in trout. *J. Exp. Zool.* **269**, 69–83.
Stifani, S., George, R. & Schneider, W.J. (1988). Solubilization and characterization of the chicken oocyte vitellogenin receptor. *Biochem. J.* **250**, 467–75.
Stifani, S., Nimpf, J. & Schneider, W.J. (1990*a*). Vitellogenesis in *Xenopus laevis* and chicken: cognate ligands and oocyte receptor. *J. Biol. Chem.* **265**, 882–8.
Stifani, S., Menn, F., Nuñez Rodriguez, J. & Schneider, W.J. (1990*b*). Regulation of oogenesis: the piscine receptor for vitellogenin. *Biochim. Biophys. Acta* **1045**, 271–9.
Tinsley, R.C., Loumont, C. & Kobel, H.R. (1996). Geographical distribution and ecology. In *The Biology of Xenopus*, ed. R.C. Tinsley & H.R. Kobel, pp. 35–59. Oxford: Oxford University Press.
Valdez Toledo, C. (1978). Maduración ovocitaria de *Bufo arenarum* adulto en función del período estacional. Doctoral dissertation, Universidad Nacional de Tucumán, Tucumán, Argentina.
Varriale, B., Pierantoni, R., Di Matteo, L., Minucci, S., Milone, M. & Chieffi, G. (1988). Relationship between estradiol-17β seasonal profile and annual vitellogenin content of liver, fat body, plasma and ovary in the frog (*Rana esculenta*) (*Gen. Comp. Endocrinol.* **69**, 328–34.
Villecco, E.I. (1998). Ovogénesis en anfibios: interacciones celulares. Doctoral dissertation, Universidad Nacional de Tucumán, Tucumán, Argentina.
Wall, D.A. & Meleka, I. (1985). An unusual lysosome compartment involved in vitellogenin endocytosis by *Xenopus* oocytes. *J. Cell Biol.* **101**, 1651–64.
Wall, D.A. & Patel, S. (1987*a*). Multivesicular bodies play a key role in vitellogenin endocytosis by *Xenopus* oocytes. *Dev. Biol.* **119**, 275–89.
Wall, D.A. & Patel, S. (1987*b*). The intracellular fate of vitellogenin in *Xenopus* oocytes is determined by its extracellular concentration during endocytosis. *J. Biol. Chem.* **262**, 14779–89.
Wallace, R.A. (1963). Studies on amphibian yolk: an analysis of the main body components of yolk platelets. *Biochim. Biophys. Acta* **74**, 505–18.
Wallace, R.A. (1970). Studies on amphibian yolk. IX. *Xenopus* vitellogenin. *Biochem. Biophys. Acta* **215**, 176–83.
Wallace, R.A. (1985). Vitellogenesis and oocyte growth in non-mammalian vertebrates. In *Developmental Biology*, vol. 1, ed. L.W. Browder, pp. 127–77. New York: Plenum Press.
Wallace, R.A. & Dumont, J.N. (1968). The induced synthesis and transport of yolk proteins and their accumulation by the oocytes in *Xenopus laevis*. *J. Cell Comp. Physiol.* **72**, 73–89.
Wallace, R.A., Misulovin, Z. & Wiley, H.S. (1980). Growth of anuran oocytes in serum supplemented medium. *Reprod. Nutr. Dev.* **20**, 699–708.
Wallace, R.A. & Selman, K. (1990). Ultrastructural aspects of oogenesis and oocyte growth in fish and amphibian. *J. Electron Microsc. Technique* **16**, 175–201.
Yoshizaki, N. & Yonezawa, S. (1994). Cathepsin D activity in the vitellogenesis of *Xenopus laevis*. *Dev. Growth Differ.* **36**, 299–306.

https://doi.org/10.1017/S0967199499000349 Published online by Cambridge University Press