Zygote **10** (May), pp 163–173. © 2002 Cambridge University Press
DOI:10.1017/S0967199402002228 Printed in the United Kingdom

Ultrastructural characteristics of the follicle cell–oocyte interface in the oogenesis of *Ceratophrys cranwelli*

Evelina I. Villecco, Susana B. Genta, 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 del Tucumán (UNT), Chacabuco 461, 4000 San Miguel de Tucumán, Tucumán, Argentina

Date submitted: 27.11.01. Date accepted: 14.1.02

Summary

In this work we carried out an ultrastructural analysis of the cell interface between oocyte and follicle cells during the oogenesis of the amphibian *Ceratophrys cranwelli*, which revealed a complex cell–cell interaction. In the early previtellogenic follicles, the plasma membrane of the follicle cells lies in close contact with the plasma membrane of the oocyte, with no interface between them. In the mid-previtellogenic follicles the follicle cells became more active and their cytoplasm has vesicles containing granular material. Their apical surface projects cytoplasmic processes (macrovilli) that contact the oocyte, forming gap junctions. The oocyte surface begins to develop microvilli. At the interface both processes delimit lacunae containing granular material. The oocyte surface has endocytic vesicles that incorporate this material, forming cortical vesicles that are peripherally arranged. In the late previtellogenic follicle the interface contains fibrillar material from which the vitelline envelope will originate. During the vitellogenic period, there is an increase in the number and length of the micro- and macrovilli, which become regularly arranged inside fibrillar tunnels. At this time the oocyte surface exhibits deep crypts where the macrovilli enter, thus increasing the follicle cell–oocyte junctions. In addition, the oocyte displays coated pits and vesicles evidencing an intense endocytic activity. At the interface of the fully grown oocyte the fibrillar network of the vitelline envelope can be seen. The compact zone contains a fibrillar electron-dense material that fills the spaces previously occupied by the now-retracted microvilli. The macrovilli are still in contact with the surface of the oocyte, forming gap junctions.

Keywords: Amphibians, Follicle, Interaction, Interface, Oogenesis

Introduction

The formation of functional gametes is a complex and highly regulated developmental process that begins in the embryo and continues until the animal is adult or sexually mature. Shortly after fertilisation of the egg and during the earliest stages of vertebrate embryogenesis a finite number of non-dividing primordial germ cells are produced. These cells eventually migrate up to the mesothelium and colonise the genital ridge, thus originating a gonad that consists of germinal and somatic cellular elements (Hardisty, 1978).

All correspondence to: S.S. Sanchez, Departamento de Biología del Desarrollo, INSIBIO (CONICET-UNT), Chucabuco 461, 4000 San Miguel de Tucumán, Tucumán, Argentina. Tel: +54 381 4247752, ext. 358. Fax: +54 381 4248025. e-mail: ssanch@unt.edu.ar

Studies from a number of biological systems have shown that soma–germ cell interactions play critical roles in both the germ line and the physiological functions of the sexually mature gonad (Gilula *et al.*, 1978; Morgan & Mahowald, 1996). In mammals, for instance, cell interactions necessary for the survival of the germ cells take place between the components of ovarian follicles (Fleischman, 1993).

Matsui *et al.* (1990) and Keshet *et al.* (1991), also working with mammals, found that somatic cells produce a stem cell factor (SCF) that interacts with the c-kit receptor on germ cells. c-kit signalling increases germ cell survival, proliferation and migration and promotes adhesion to somatic gonadal cells (Dolci *et al.*, 1991, 1993; Marziali *et al.*, 1993; Pesce *et al.*, 1997).

Anderson & Albertini (1976) noted the presence of gap junctions between the granulosa cells and the oocyte. The results obtained with mutational analyses

led Simon *et al*. (1997) to suggest that intercellular signalling, important for oogenesis and ovulation, passes through these junctions.

In the anuran amphibian species *Bufo arenarum*, which has an annual reproductive cycle, the presence of gap junctions, their hormonal regulation and their involvement in meiotic arrest have been determined in fully grown oocytes (Villecco *et al*., 1996, 2000).

Amphibians are an appropriate biological model to study the interactions that take place in ovarian follicles since the follicles pass through different periods (previtellogenic, vitellogenic and maturation) during which various processes related to oocyte growth and maturation occur.

In the present work we analysed, by means of a structural analysis, the cell interactions between the oocyte and follicle cells of the anuran amphibian *Ceratophrys cranwelli* (family Leptodactilidae) during the different periods of oogenesis, since structural features are likely to underlie follicle cell–oocyte intercellular signalling.

The selection of *Ceratophrys cranwelli* was made on the basis that this species is capable of effecting rapid oogenesis when stimulated by strong transitory rainfalls, a factor that seems to be responsible for the activation of the reproductive process (Tinsley *et al*., 1996). This accelerated process might require the active participation of the oocyte surface and follicular epithelium, causing ultrastructural changes that allow a better observation of the way in which interactions take place.

Materials and methods

Animals

Ceratophrys cranwelli ovarian follicles from specimens recently collected in the neighbourhood of Tucumán (Argentina) during the spring–summer period were used.

Tissue preparation for microscopy

Sexually mature females were anaesthetised. After laparoscopy, mesovaries were carefully dissected to obtain isolated oocytes at different stages (Villecco, 1998), wrapped in their follicle. Isolated follicles were measured with an eyepiece micrometer in a stereoscopic microscope.

Transmission electron microscopy

For thin sectioning, whole follicles were fixed for 4 h at 4 °C in 2.5% glutaraldehyde in 0.1% M sodium phosphate (pH 7.4). Follicles were then 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 a Potter Blum MTI ultramicrotome. Slices were stained with lead citrate and uranyl acetate. Preparations were examined with a Zeiss EM 109 electron microscope.

Radioactive labelling

Pieces of ovary with five or six follicles obtained from the proximal region of the ovary (previtellogenic follicles) were incubated in antibiotic Ringer solution (25 µg/ml penicillin and 50 µg/ml streptomycin) containing 100 µCi/ml of [^{3}H]adenosine (specific activity 36.7 Ci/mM; New England Nuclear) in a total volume of 500 µl. The follicles were incubated for 5 h in a wet chamber at 20–25 °C.

Histological procedures

After incubation, follicles were fixed in Ancel & Vintemberger solution (Ancel & Vintemberger, 1948), dehydrated and embedded in paraffin–celloidin, according to Manes & Nieto (1983). Sections 4–6 µm thick, serially obtained from blocks, were attached to slides.

Autoradiography

Autoradiography was carried out with Ilford K2 liquid emulsion diluted 1:1 with distilled water. Exposure was effected for 15 days at 4 °C. Exposed slides were developed with Kodak D19 developer, washed and fixed with sodium thiosulfate. Dried slides were stained with haematoxylin–eosin and mounted for light microscopic observation. Blanks of no radioactive slides were also run.

Dye transfer

Oocytes were microinjected with a 1% aqueous solution of the fluorescent dye Lucifer Yellow CH (MW 457.2) with glass capillary micropipettes while being observed with a stereomicroscope. The injected volume was 50 nl. Injection of a follicle group was made at room temperature. To evaluate dye transfer to follicle cells, oocytes were manually defolliculated 2 h after injection with watchmakers' forceps. Fluorescent images of dye distribution were taken using Ilford HP5 (ASA 400).

Results

Ovaries

In adult females, before mating, ovaries appear as two widely developed lateral masses which, extending as far as the mid-plane, cover most of the abdominal viscera. Their surface is shiny and shows by transparency a granular structure produced by countless spherical oocytes at different stages of development. Macroscopic and stereomicroscopic observation reveals two zones: a proximal zone, followed by the mesovary, which contains previtellogenic and early vitellogenic oocytes, and a distal zone with vitellogenic and fully grown oocytes. Each ovary has about 13–16 lobes with an interlobular vein and artery between them (Fig. 1). The ovarian interlobular arteries arborise extensively and form capillary networks in the theca.

Interface of early previtellogenic oocytes

The cytoplasm of the follicle cells of these oocytes has a fair amount of ribosomes as well as a few cisterns of rough endoplasmic reticulum (Fig. 2).

Ultrastructural analysis of the interface between the follicle cells and the oocyte showed that the plasma membrane of the former is in close apposition to the oocyte plasma membrane. They become separated at intervals, forming an interface made up of small spaces with a homogeneous material of low electron density. There are no signs of an exchange of material between the follicle cells and the young oocyte at this stage of oogenesis (Fig. 2*A*). At several points where the intercellular space is not yet formed, the two plasma membranes show thickenings that resemble desmosome-like junctions.

The oolemma has a prominent glycocalyx different

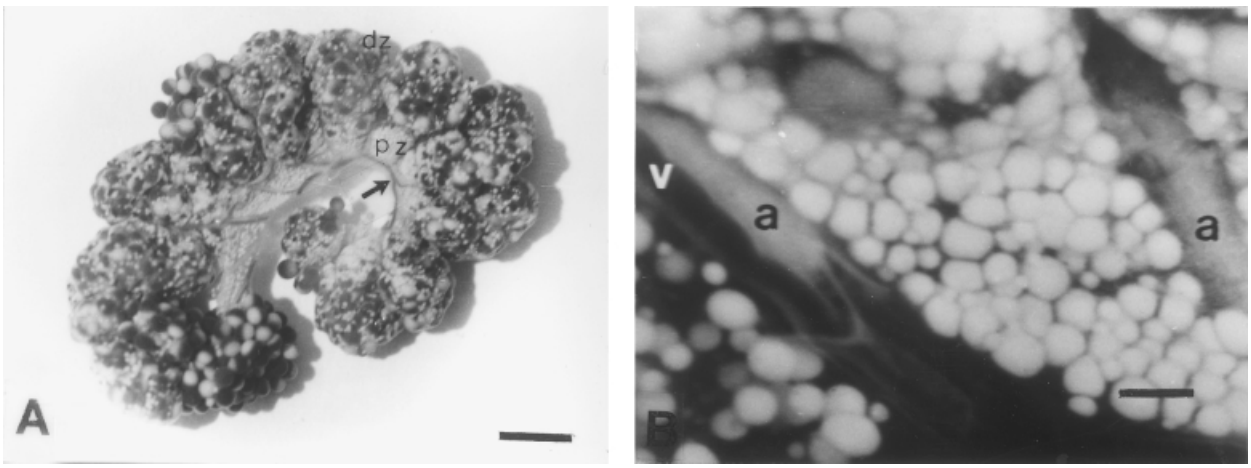

Figure 1 Anatomical characteristics of the *Ceratophrys cranwelli* ovary. (*A*) Stereoscope micrograph of the whole ovary showing 13 lobes. pz, proximal zone; dz, distal zone. The lobes are separated by branches of the ovarian artery and vein (arrow). Scale bar represents 4000 µm. (*B*) Proximal zone of the lobe limited by the interlobal vein (v) and artery (a), containing previtellogenic oocytes of different sizes. Scale bar represents 600 µm.

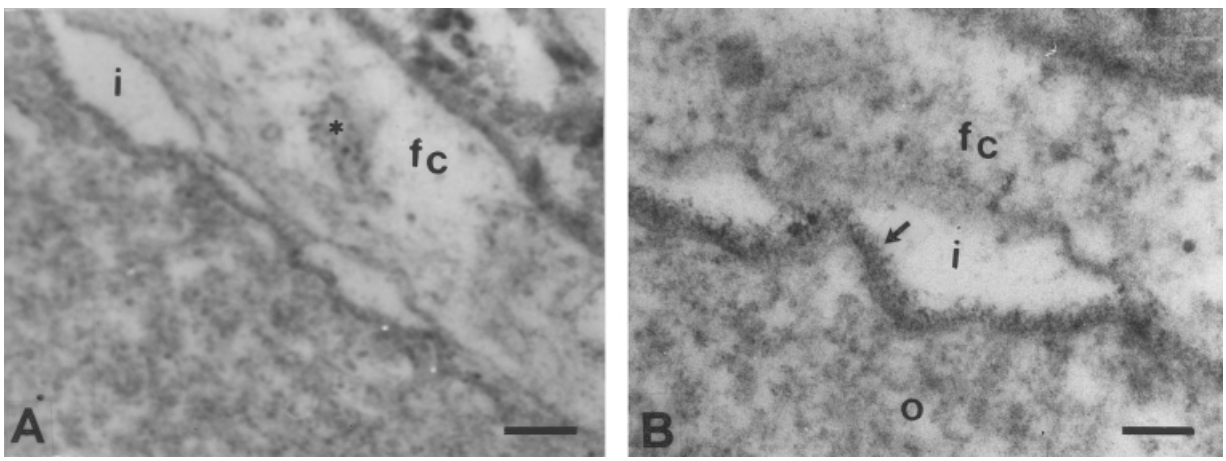

Figure 2 Electron micrographs of *C. cranwelli* early previtellogenic follicles showing the follicle cell–oocyte interface. (*A*) The cytoplasm of the follicle cells (fc) has a fair amount of ribosomes and rough endoplasmic reticulum (asterisk). o, oocyte; i, interface. Scale bar represents 0.25 µm. (*B*) The oocyte plasma membrane has a prominent glycocalyx (arrow). fc, follicle cells; o, oocyte cortex. Scale bar represents 0.16 µm.

from the plasma membrane of the follicle cells (Fig. 2*B*).

The cytoplasmic cortex of these oocytes is devoid of organelles except for some scattered mitochondria, a few small Golgi complexes, sparsely distributed cisterns of rough and smooth endoplasmic reticulum and numerous ribosomes. No signs of yolk production are evident.

Interface of mid-previtellogenic oocytes

The follicle cells of mid-previtellogenic oocytes are metabolically very active. These cells are characterised by their electron-dense cytoplasm filled with rough endoplasmic reticulum, free ribosomes and glycogen (Fig. 3*A*, *B*). Near the apical surface in their cytoplasm are vesicles that contain two types of particles: one

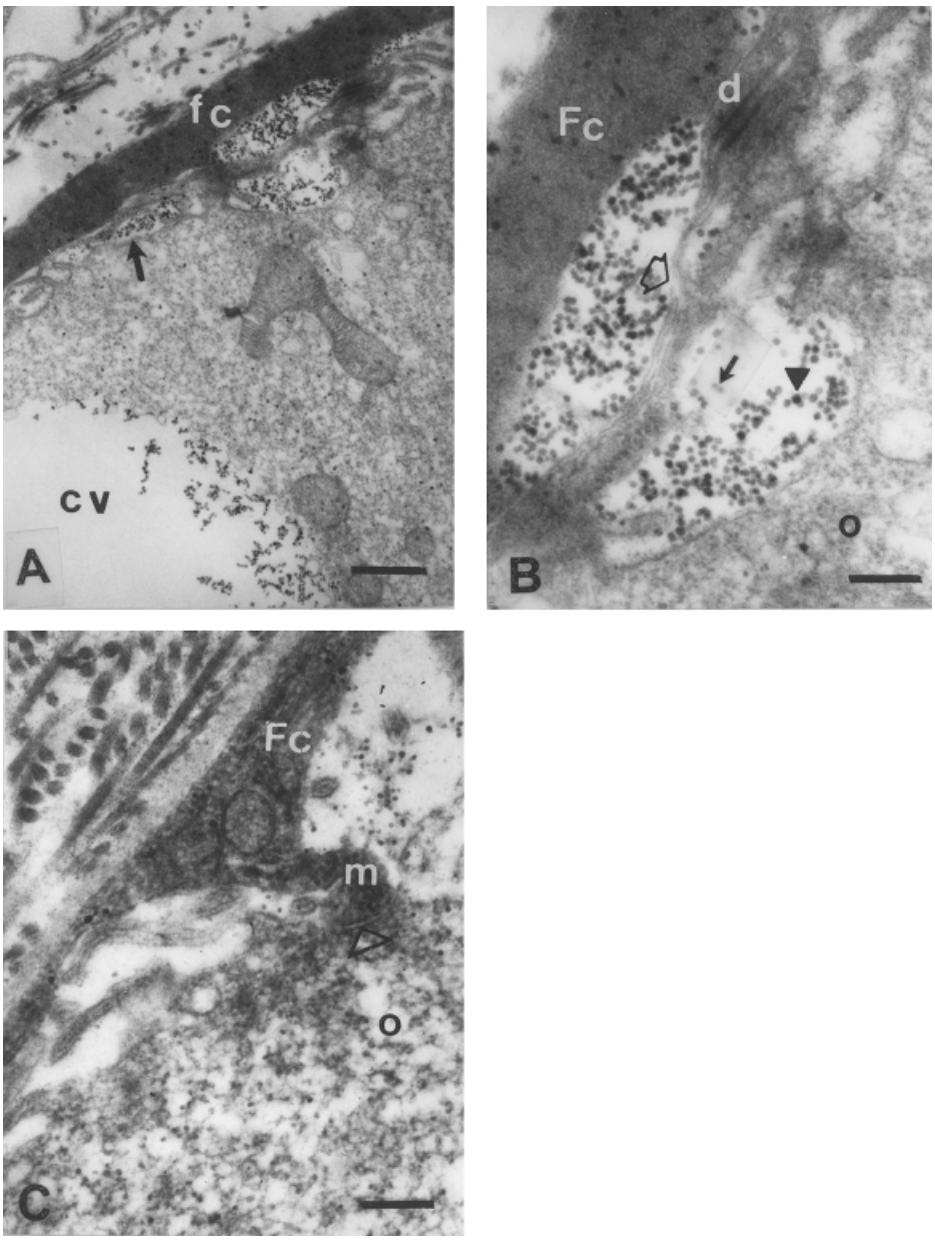

Figure 3 Electron micrographs of *C. cranwelli* mid-previtellogenic follicles showing the follicle cells–oocyte interface. (*A*) Follicle cell (fc) with abundant free ribosomes and glycogen. At the interface (arrow) the micro- and macrovilli delimit lacunae containing granular material. The oocyte cortex shows cortical vesicles (cv) with the same material as the interface. Scale bar represents 0.65 µm. (*B*) Follicle cell with a vesicle containing two types of particles: one of 15–25 nm (arrow) and another of 25–50 nm (arrowhead). The open arrow shows discontinuous membranes. The follicle cells have desmosomes (d) between the macrovilli. The oocyte (o) surface has invaginations with the same particulate material. Scale bar represents 0.24 µm. (*C*) The follicle cell (Fc) displays a macrovillus (m) that passes through the interface and contacts the oocyte (o), forming a gap junction (open arrowhead). Scale bar represents 0.30 µm.

ranging from about 15 to 25 nm and the other from 25 to 50 nm, as well as fibrillar material that connects the smaller particles. The vesicles are separated from the interface by a thin discontinuous cytoplasm (Fig. 3*B*).

Extending from the margin of their apical surface, follicle cells exhibit long processes called macrovilli, many of which penetrate into the interface and make contact with the developing oocytes, often ending in morphologically specialised junctions such as gap junctions. These have smooth plasma membranes with a narrow (<2 nm) extracellular space between them that are a little more electron-dense than non-junctional plasma membranes (Fig. 3*C*). Desmosomes were also observed between the macrovilli as well as between the macrovilli and the oocyte (Fig. 3*B*).

The oocyte surface displays only a few scattered short microvilli that differ from the macrovilli in that they are thinner and of a lower electron density. At the interface, both micro- and macrovilli delimit lacunae containing granular material. The oocyte surface also has endocytic invaginations that incorporate this material, forming peripherally arranged cortical vesicles ranging from about 2 µm to 3 µm in diameter (Fig. 3*A*).

For the purpose of finding out whether the follicle cells from previtellogenic oocytes are actively involved in the synthesis of nucleic acids, follicles were incubated with [^{3}H]adenosine, a precursor of nucleic acid synthesis. Fig. 4 shows the autoradiograph of a previtellogenic follicle incubated with the precursor for 5 h, in which the follicle cells display strong radioactivity.

Interface of late previtellogenic oocytes

At the interface of late previtellogenic oocytes fibrillar material begins to appear. This material is first evident as fine filaments in isolated patches that become continuous, forming a strip between the macro- and the microvilli sections. Particles were not observed.

The oocyte surface had a different appearance from that observed in previous stages and no endocytic invaginations could be seen (Fig. 5). The cortex of these oocytes displays cortical vesicles regularly arranged, with particulate material similar to that present in mid-vitellogenic oocytes (Fig. 5).

Interface of vitellogenic follicles

The cytoplasm of the follicle cells of vitellogenic oocytes has vesicles, a fair amount of smooth endoplasmic reticulum, ribosomes, mitochondria and lipid droplets. The nuclei show evenly distributed heterochromatin. The interface, with a uniform thickness of 2.6 µm, is formed by a fibrillar pattern within which macro- and microvilli are arranged (Fig. 6*A*). This pattern, which is the precursor of the vitelline envelope, is made up of bundles of fibres of average electron density arranged in four-walled tunnels inside which sections of macro- and microvilli can be observed. Fig. 6*C* shows a perpendicular section of the interface in which microvilli, about 2 µm long, penetrate into the tunnels and extend inside them, occupying the central region without reaching the subfollicular space. Fig. 6*B* shows a cross-section of the interface in which the tunnels formed by the fibrillar pattern are arranged in a net in the spaces of which macro- and microvilli, with a diameter of 0.15 and 0.05 µm respectively, have a round shape. The three-dimensional arrangement of this pattern can be seen in the inset of Fig. 6*C*. The structure of the net becomes more open and irregular in the area close to the follicle cells (Fig. 6*A*).

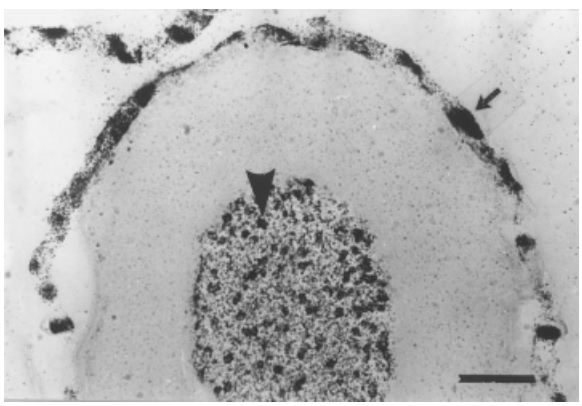

Figure 4 Autoradiography of a section through a previtellogenic oocyte of *C. cranwelli* incubated for 5 h in [^{3}H]adenosine. The follicle cells (arrow), the oocyte nucleoli (arrowhead) and the nucleoplasm display an intense radioactivity. Scale bar represents 20 µm.

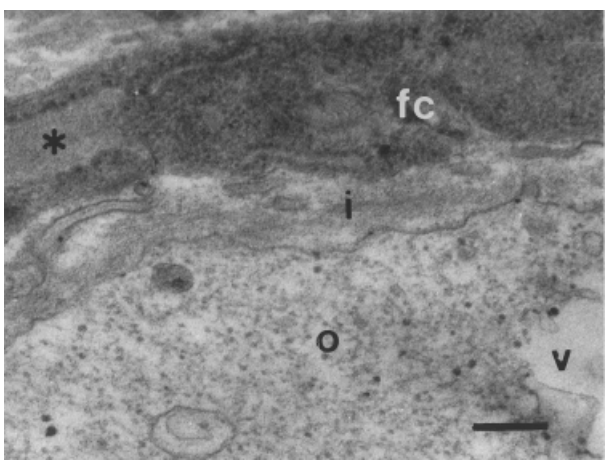

Figure 5 Electron micrograph of a *C. cranwelli* late previtellogenic follicle showing the follicle cells–oocyte interface. The follicle cell (fc) has abundant ribosomes, rough endoplasmic reticulum and vesicles with fibrillar material (asterisk). The interface (i) shows the same material between the macro- and microvilli. The oocyte cortex (o) displays a cortical vesicle (v). Scale bar represents 0.50 µm.

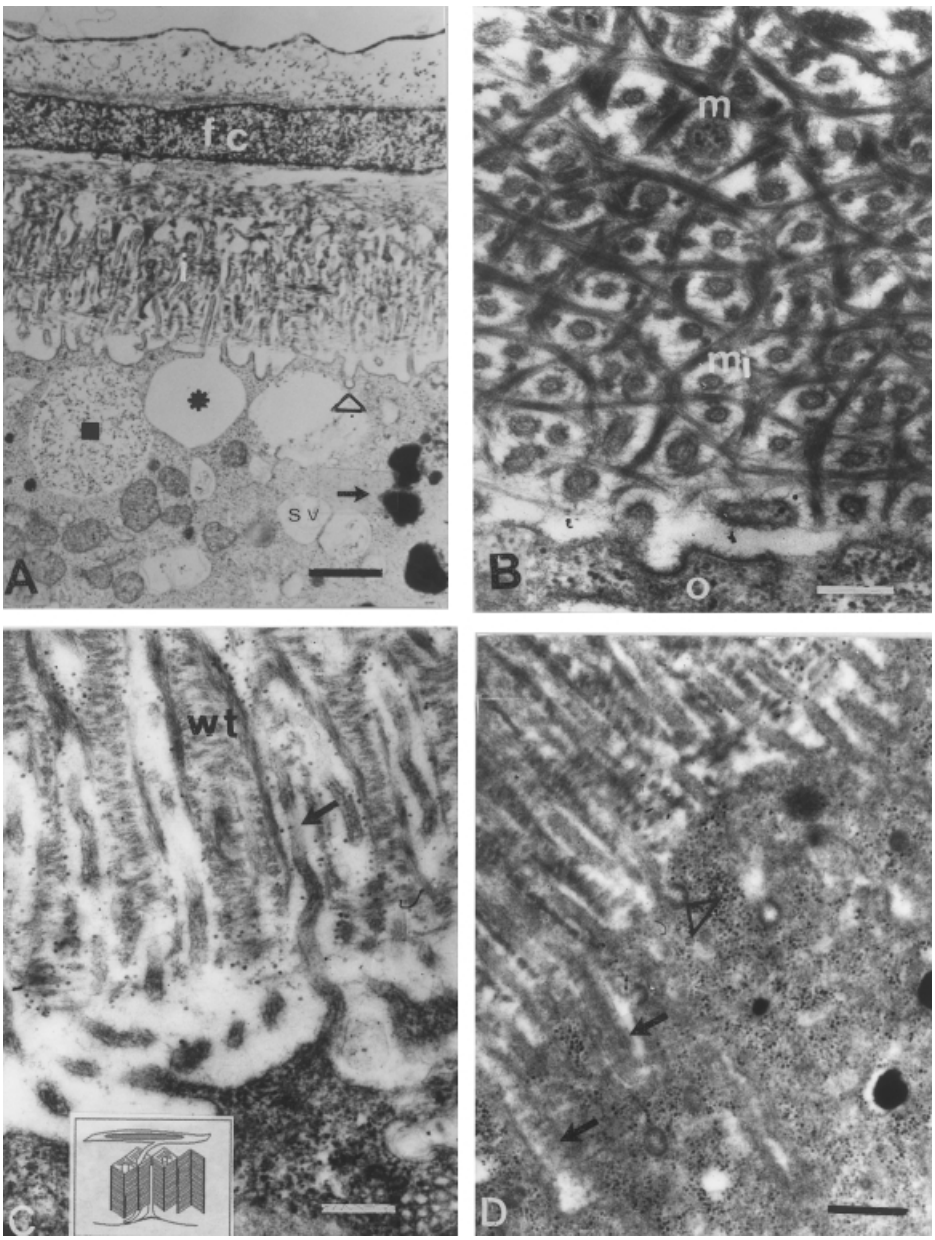

Figure 6 Electron micrograph of *C. cranwelli* vitellogenic follicles showing the follicle cell–oocyte interface. (*A*) The follicle cell (fc) nuclei show evenly distributed heterochromatin. The interface (i), with a uniform thickness of 2.6 µm, presents a fibrillar pattern within which the macro- and microvilli are arranged. The cortex of these oocytes shows some cortical vesicles with numerous particles (filled square) and some empty ones (asterisk). Arrowhead indicates coated pits. The subcortical cytoplasm shows smaller vesicles (sv), vitelline platelets (arrow) and mitochondria. Scale bar represents 1.3 µm. (*B*) Cross-section of the follicle cell–oocyte interface showing the tunnel arranged in a net in the spaces of which macrovilli (m) and microvilli (mi) can be seen. Scale bar represents 0.28 µm. (*C*) Perpendicular section of the follicle cell–oocyte interface showing double-walled tunnels (wt) and the microvilli (arrow) penetrating inside them. Scale bar represents 0.52 µm. (*D*) Follicle cell–oocyte interface in late vitellogenesis showing an increase in the number of macro- and microvilli. At the oocyte surface, crypts into which the macrovilli (arrow) penetrate can be seen. Arrowhead indicates coated pits. Coated vesicles are also present. Scale bar represents 0.52 µm.

In Fig. 6*A* the micrograph of these oocytes shows cortical vesicles, some with numerous particles, some with both particles and membranes, and some empty ones. The subcortical cytoplasm contains numerous vesicles, smaller but with the same content as the cortical vesicles. Between these the first vitelline platelets appear, indicating the onset of vitellogenesis.

As the process advances, the interface of the mid-vitellogenic and of the late vitellogenic oocytes show changes related to it, including an increase in the number of macro- and microvilli (Fig. 6*D*).

The surface of the oocyte now contains crypts and

protuberances with numerous coated pits and coated vesicles (Fig. 6*D*). Microvilli project from the protuberances and often exhibit coated pits and coated vesicles. Macrovilli enter the crypts, gap junctions, desomosomes and adherent junctions being formed where they contact the oocyte (Fig. 6*D*).

The interface of fully grown follicles

In fully grown oocytes, the follicular epithelium exhibits star-shaped follicle cells with lateral projections that contact those of neighbouring cells. The spaces between them, called channels, would be a direct pathway from the blood capillaries along the tunnels of the vitelline envelope towards the oocyte. Macrovilli continue contacting the oocyte surface, at which level gap junctions appear.

The functional nature of these junctions was determined by injecting the oocyte with Lucifer Yellow. The isolated fluorescent follicular epithelium can be seen in Fig. 7. It should be noted that these oocytes, when collected from a hibernating female, are not functional.

In the interface the fibrillar network of the vitelline envelope is divided in two zones: a loose one near the follicle cells and a more compact one near the oocyte (Fig. 8*A*). The vitelline envelope shows a fibrillar electron-dense material that causes a decrease in the width of the tunnels (Fig. 8*B*), electron-dense particles being observed in the reduced space (Fig. 8*C*).

The perivitelline space also exhibits dense particles while microvilli, which have become scarcer, are shorter and thicker.

Discussion

Analysis of the oocyte–follicle cell interface during the oogenesis of *Ceratophrys cranwelli* revealed the development of a complex cell–cell interaction that changes throughout the various oogenetic periods. This finding is in agreement with the fact that oogenesis can be regarded as an interdependent process in which the follicle cells and the oocyte pass through several stages, with signals being exchanged sequentially to facilitate the progress of both from one step to the next. Moreover, oocytes and follicle cells may be controlled independently by factors that act on both of them to achieve a parallel development.

The ovaries of *C. cranwelli* specimens at the time of their capture contained oocytes at all developmental stages. The previtellogenic and early vitellogenic oocytes are located in the proximal region and the vitellogenic and fully grown oocytes in the distal region, in contrast to reports concerning the ovary of *Rana pipiens* (Kemp, 1956). *Xenopus laevis* (Dumont, 1972) and *Bufo arenarum* (Valdez & Pisano, 1980). In *C. cranwelli*, after ovulation, a large number of previtellogenic and early vitellogenic oocytes are available to continue their development and originate a new wave of fully grown oocytes.

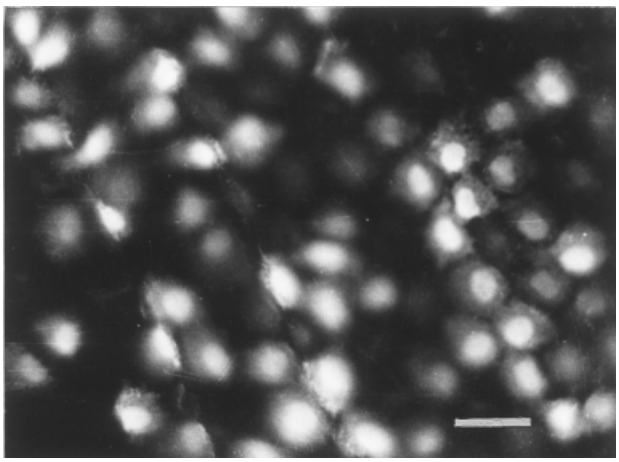

Figure 7 Dye transfer from the oocyte to follicle cells during the breeding season. Image of a follicle cell preparation examined by fluorescence after Lucifer Yellow injections into an oocyte. Scale bar represents 20 μm.

In the smaller previtellogenic follicles the follicle cells and oocytes exhibit few cytoplasmic differentiations, no exchange of particles between the two types of cells being observed. The follicular epithelium does not have a well-defined basal membrane, probably due to the fact that the follicle is becoming organised since, in amphibians, oogonia remain in the adult ovary and continually enter meiosis, turning into oocytes (Tokarz, 1978). The abundant glycocalyx present in the oocytes agrees with the idea that during folliculogenesis ligand–receptor interactions would be activated. As suggested for *Xenopus laevis*, neutrophins would be involved in this process (Ojeda *et al*., 1992). In contrast to these follicles, mid-previtellogenic follicles exhibit a follicular epithelium composed mainly of metabolically very active cells. These cells incorporate large amounts of [^{3}H]adenosine, as seen in autoradiographs and transmission electron microscopic observations that show a cytoplasm with large amounts of free ribosomes, glycogen, rough endoplasmic reiticulum and particle-filled vesicles, indicating great cellular activity. This activity agrees with that observed by Callebau (1991) in avian ovarian follicles containing granulosa cells. The presence in these follicles of an interface with numerous particles of the same nature as that observed inside the vesicles of the follicles cells, added to the discontinuity of the cytoplasm that limits such vesicles, suggests a transfer of these particles to the interface. Moreover, micrographs (Fig. 3*B*) show that the oocyte membrane forms invaginations that incorporate the interface particles. In the oocyte cortex, these investigations form cortical vesicles with particulate material

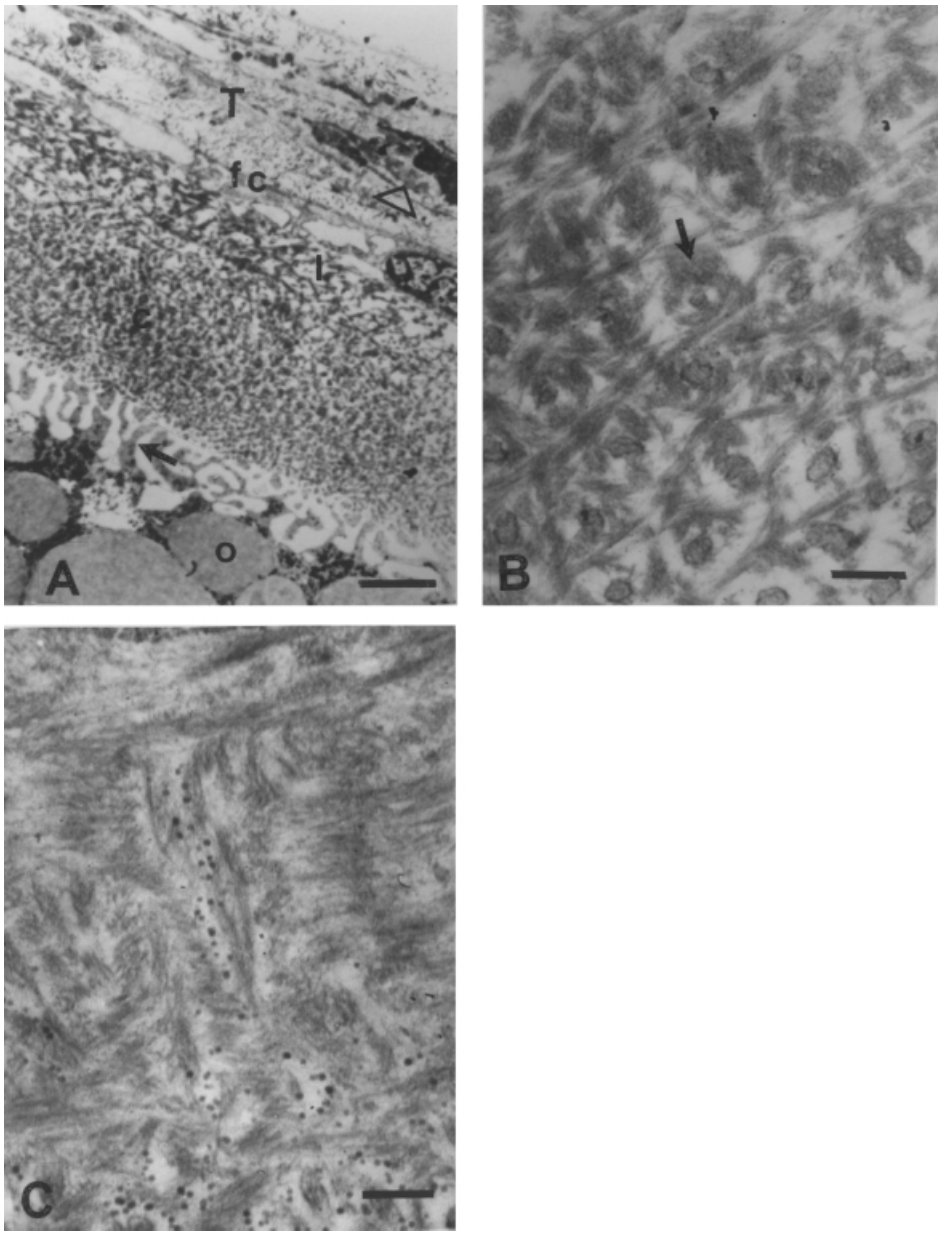

Figure 8 Electron micrographs of *C. cranwelli* fully grown follicles showing the follicle cell–oocyte interface. (*A*) At the interface the fibrillar network of the vitelline envelope has two zones: a loose one (l) and a more compact one (c). The microvilli are shorter and thicker (arrow). fc, follicle cells; t, theca; o, oocyte; open arrowhead, capillary. Scale bar represents 1.98 µm. (*B*) Cross-section of the follicle cell–oocyte interface showing fibrillar material (arrow) that closes the tunnel of the vitelline envelope. Scale bar represents 0.29 µm. (*C*) Perpendicular section of the interface showing a decrease in the width of the tunnels and particles inside them. Scale bar represents 0.29 µm.

and membranes, this being an outstanding characteristic of this species. These cortical vesicles, the formation of which is a gradual process as evidenced by the analysis of previtellogenic and early vitellogenic oocytes, were not observed in the species studied up to now: *Xenopus laevis* (Dumont, 1972), *Flectonotus pygmaeus* (del Pino & Humphries, 1978), *Gastrotheca riobambae* (del Pino *et al*., 1986) and *Bufo anenarum* (Valdez & Pisano, 1980).

Ultrastructural analyses would point to the existence of an intense interaction that takes place through the interface which, like the intercellular bridges in insects (Giorgi, 1978), allows the passage of particles as large as ribosomes. These particles, possibly involved in the control of oogenesis and in embryonic development, would be protected inside the cortical vesicles until their expression, originating specific domains in the oocytes (Carotenuto *et al*., 2000).

The fact that the changes in the follicular epithelium can be related to changes in the interface and the cortex

of the oocyte, which becomes competent to incorporate the above particles, would indicate a coordination in the process of formation of the cortical vesicles. While this interaction is taking place, gap junctions are present, probably transmitting signals that might coordinate these cellular interactions.

In late previtellogenic oocytes follicle cells stop releasing particles and in their cytoplasm we can see vesicles with a fibrillar material similar to the one that appears in the interface, which is a precursor of the vitelline envelope. This fact demonstrates the participation of the follicular epithelium in the formation of the vitelline envelope, in agreement with the findings of Dumont & Brummet (1978) in *Xenopus laevis*, and of Cabada *et al*. (1996) in *Bufo arenarum*.

Although the oocyte surface has no traces of the endocytic invaginations seen in the mid-previtellogenic follicles, the cortical vesicles are regularly arranged in the cortex, which would indicate that their formation belongs to a previous stage.

The images of vitellogenic follicles reveal an intense activity in the area of interaction between the follicular epithelium and the oocytes, related to the vitellogenic process. At the apical surface of the follicle cells there is a significant increase in macrovilli, which penetrate the vitelline envelope and contact the outer surface of the oocyte, forming numerous gap junctions and desmosomes that are important interaction sites. At the same time, the oocyte surface exhibits numerous microvilli and deep crypts, coated pits and vesicles.

It is known that FSH-like gonadotropins promote vitellogenesis through the mediation of estradiol synthesised by follicle cells (Redshaw, 1972; Wallace, 1985). Moreover, gonadotrophins promote the uptake of yolk proteins by vitellogenic oocytes, this effect being apparently mediated by follicle cells through an unknown mechanism (Wallace & Bergink, 1974).

Since vitellogenin uptake by the oocytes has proved to be a hormone-dependent process that requires the presence of the follicular epithelium, a possibility to be considered is whether the acquisition of endocytic competence could be transferred to the oocyte via gap junctional contacts with the overlying follicle cell epithelium. Interestingly, in this regard, we have demonstrated in *Bufo arenarum* (Villecco *et al*., 1996) and in *Ceratophrys cranwelli* (Villecco, unpublished data) that the FSH-like hormone induced gap junction coupling between the follicle cells and the oocyte. We have also demonstrated in *Bufo arenarum* oocytes that these junctions are involved in meiotic arrest caused by the transfer of cAMP, probably as a result of the effect of FSH acting on the follicle cells.

It seems likely that other junctions such as desmosomes would be involved in follicle cell–oocyte interactions. Desmosomes provide a stable contact between follicle cells and the oocyte and might constitute membrane domains at the level of which exocytosis and signal transduction processes could take place.

At the interface of fully grown oocytes the totally formed vitelline envelope can be observed. Microvilli are retracted and the fibrillar material partly closes the tunnels through which the microvilli passed. This would indicate that microvilli perform their function mainly during the vitellogenic process.

From an ontogenetic viewpoint the fibrillar material of the vitelline envelope in amphibians may have originated mainly for the purpose of arranging the increasingly large number of oocyte microvilli so as to keep them perpendicular to the surface of oocyte, thus increasing the absorption area. In coelomic eggs, the vitelline envelope changes its structural characteristics in order to prevent sperm penetration and consequent fertilisation (Elinson, 1973; Katagiri, 1974). In eggs that have passed through the initial segment of the oviduct the vitelline envelope changes again, increasing the probability of successful fertilisation (Miceli *et al*., 1978; Yoshizacki & Katagiri, 1982), and at the time of fertilisation it shows changes related to the cortical reactions capable of forming a block to polyspermy (Wyrick *et al*., 1974; Grey *et al*., 1976).

Macrovilli remain and contact the oocyte, forming gap junctions as revealed by transmission electron microscopic observations and by the passage of Lucifer Yellow from the oocyte to the follicle cells. We have also found that gap junction communications during winter appear to be suppressed, as indicated by the blocking of the passage of the dye (Villecco, unpublished data).

The presence of functional gap junctions in fully grown oocytes of *Ceratophrys cranwelli* in the breeding season suggests their possible role in meiotic arrest, as demonstrated for *Bufo arenarum* (Villecco *et al*., 1996, 2000).

The passage of particles observed in the tunnels, now extremely reduced, of the vitelline envelope demonstrates that their interactions through the interface still exist.

In conclusion, in the follicles of *Ceratophrys cranwelli* the follicle cell–oocyte interface, which varies throughout the various oogenetic periods, shows intense follicle cell–oocyte interactions. These take place through the passage of particles from the follicle cells to the oocyte and through the gap junctions. The former process leads to the formation of cortical vesicles and the gap junctions would allow the passage of regulatory molecules such as cAMP, which would participate in meiotic arrest as well as in the acquisition of endocytic competence.

Acknowledgements

This work was supported by a PID from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT). The authors thank Mr E. Dozetos for his photographic assistance, and Mrs Virginia Méndez for her proofreading.

References

Ancel, P. & Vintembenger, P. (1948). Recherches dur le determinisme de la symétrie bilatérale dans l'oeuf des amphibiens. *Bull. Biol. Fr. Belg. Suppl.* **31**, 1–181.

Anderson, E. & Albertini, D.F. (1976). Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *J. Cell Biol.* **71**, 680–6.

Cabada, M.O., Sanchez Riera, A.N., Genta, H.D., Sanchez, S.S. & Barisone, G.A. (1996). Vitelline envelope formation during oogenesis in *Bufo arenarum*. *Biocell* **20**, 77–86.

Cellebaut, M. (1991). Pyriform-like and holding granulosa cells in the avian ovarian follicle wall. *Eur-Arch. Biol.* (Bruxelles) **102**, 135–45.

Carotenuto, R., Vaccaro, M.C., Capriglione, T., Petrucci, T.C. & Campanella, C. (2000). α-Spectrin has a stage-specific asymmetrical localization during *Xenopus* oogenesis. *Mol. Reprod. Dev.* **55**, 229–39.

Del Pino, E.M. & Humpries, A.A., Jr (1978). Multiple nuclei during early oogenesis in *Flectonotus pygmaeus* and other marsupial frogs. *Biol. Bull.* **154**, 198–212.

Del Pino, E.M., Steibeisser, H., Hofmann, A., Dreyer, C., Campos, M. & Trendelenburg, M.F. (1986). Oogenesis in the egg-brooding frog *Gostrotheca riobambae* produces large oocytes with fewer nucleoli and low RNA content in comparison to *Xenopus laevis*. *Differentiation* **32**, 24–33.

Dolci, S., Williams, D.E., Ernst, M.K., Resnick, J.L., Braman, C.I., Fock, L.F., Lyman, S.D., Boswell, S.H. & Donovan, P.J. (1991). Requirements for mast cell growth factor for primordial germ cell survival in culture. *Nature* **352**, 809–11.

Dolci, S., Pesce, M. & De Felici, M. (1993). Combined action of stem cell factor, leukemia inhibitory factor, and cAMP on *in vitro* proliferation of mouse primordial germ cells. *Mol. Reprod. Dev.* **35**, 134–9.

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

Dumont, J.N. & Brummet, A.R. (1978). Oogenesis in *Xenopus laevis* (Daudin). V. Relationships between developing oocytes and their investing follicular tissues. *J. Morphol.* **155**, 73–98.

Elinson, R.P. (1973). Fertilization of frog body cavity eggs enhanced by treatments affecting the vitelline coat. *J. Exp. Zool.* **183**, 291–302.

Fleischman, R.A. (1993). From white spots to stem cells: the role of the Kit receptor in mammalian development. *Trends Genet.* **9**, 285–90.

Gilula, N.B., Epstein, M.L. & Beers, W.H. (1978). Cell-to-cell communication and ovulation: a study of the cumulus–oocyte complex. *J. Cell Biol.* **78**, 58–75.

Giorgi, F. (1978). Intercellular bridges in ovarian follicles of *Drosophila melanogaster*. *Cell Tissue Res.* **186**, 413–22.

Grey, R.D., Working, N.G. & Hedrick, J.L. (1976). Evidence that the fertilization envelope blocks sperm entry eggs of *Xenopus laevis*: interaction of sperm with isolated envelopes. *Dev. Biol.* **54**, 52–60.

Hardisty, M.W. (1978). Primordial germ cells and the vertebrate germ line. In *The Vertebrate Ovary* (ed. R.E. Jones), pp. 1–45. New York: Plenum Press.

Katagiri, C. (1974). A high frequency of fertilization in premature and mature coelomic toad eggs after enzymic removal of the vitelline membrane. *J. Embryol. Exp. Morphol.* **31**, 573–81.

Kemp, N.E. (1956). Electron microscopy of *Rana pipiens* oocytes. *J. Biophys. Biochem. Cytol.* **2**, 282–91.

Keshet, E., Lyman, S.D., Williams, D.E., Anderson, D.M., Jenkins, N.A,. Copeland, N.G. & Parada, L.F. (1991). Embryonic RNA expression patterns of the c-kit receptor and its cognate ligand suggest multiple functional roles in mouse development. *EMBO J.* **10**, 2425–35.

Manes, M.E. & Nieto, O.L. (1983). A fast and reliable celloidin-paraffin embedding technique for yolked amphibian embryos. *Mikroskopie* **40**, 341–3.

Marizali, B., Lazzaro, D. & Sorrentino, V. (1993). Binding of germ cells to mutant Sld Sertoli cells is defective and is rescued by expression of the transmembrane form of the c-kit ligand. *Dev. Biol.* **157**, 182–90.

Matsui, Y., Zsebo, K.M. & Hogan, B.L.M. (1990). Embryonic expression of a haematopoietic growth factor encoded by the S1 locus and the ligand for c-kit. *Nature* **347**, 666–9.

Miceli, D.C., Fernandez, S.N., Raisman, J.S. & Barbieri, F.S. (1978). A trypsin-like oviductal proteinase involved in *Bufo arenarum* fetilization. *J. Embryol. Exp. Morphol.* **48**, 79–91.

Morgan, M.M. & Mahowald, A.P. (1996). Multiple signaling pathways establish both the individuation and the polarity of the oocyte follicle in *Drosophila*. *Arc. Insect Biochem. Physiol.* **33**, 211–30.

Ojeda, S.R., Dissen, G.A., Malamed, S. & Hirshfield, A.N. (1992). A role for neurotrophic factors in ovarian development. In *Ovarian Cell Interactions* (ed, A.J.W. Hsueh & D. Schomberg), pp. 181–202, Norwell, MA: Symposia.

Pesce, M., Di Carlo, A. & De Felici, M. (1997). The c-kit receptor is involved in the adhesion of mouse primordial germ cells to somatic cells in culture. *Mech. Dev.* **68**, 37–44.

Redshaw, M.R. (1972). The hormonal control of the amphibian ovary. *Am. Zool.* **12**, 289–306.

Simon, A.M., Goodenough, D.A., Li, E. & Paul, D.L. (1997). Female infertility in mice lacking connexin 37. *Nature* **385**, 525–9.

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. 35–9. Oxford: Oxford University Press.

Tokarz, R.R. (1978). Oogonial proliferation, oogenesis and folliculogenesis in nonmammalian vertebrates. In *The Vertebrate Ovary* (ed. R.R. Jones), pp. 145–79. New York: Plenum Press.

Valdez Toledo, C.L. & Pisano, A. (1980). Fases ovogenéticas en *Bufo arenarum*. *Reproducción* **4**, 315–30.

Villecco, E.I. (1998). Ovogénesis en anfibios: interacciones

cellulares. Doctoral dissertation, Universidad Nacional de Tucumán, Tucumán, Argentina.

Villecco, E.I., Aybar, M.J., Sánchez, S.S. & Sánchez Riera, A.N. (1996). Heterologous gap junctions between oocyte and follicle cells in *Bufo arenarum*: hormonal effects on their permeability and potential role in meiotic arrest. *J. Exp. Zool.* **276**, 76–85.

Villecco, E.I., Aybar, M.J., Genta, S.B., Sánchez, S.S. & Sánchez Riera, A.N. (2000). Effect of gap junction uncoupling in full *Bufo arenarum* ovarian follicles: participation of cAMP in meiotic arrest. *Zygote* **8**, 171–9.

Wallace, R.A. (1985). Vitellogenesis and oocyte growth in nonmammalian vertebrates. In *Developmental Biology*, vol. 1 (ed. L.W. Browder), pp. 127–77. New York: Plenum Press.

Wallace, R.A. & Bergink, E.W. (1974). Amphibian vitellogenin: properties, hormonal regulation of hepatic synthesis and ovarian uptake, and conversion to yolk proteins. *Am. Zool.* **14**, 1159–75.

Wyrick, R.E., Nishihara, T. & Hedrick, J.L. (1974). Agglutination of jelly coat and cortical granule components and the block to polyspermy in the amphibian *Xenopus laevis*. *Proc. Natl. Acad. Sci. USA* **71**, 2067–71.

Woodruff, R.I. & Tiney, L.G. (1998). Intercellular bridges between epithelial cells in the *Drosophila* ovarian follicle: a possible aid to localized signaling. *Dev. Neural Biol.* **200**, 82–91.

Yoshizaki, N. & Katagiri, C. (1982). Acrosome reaction in sperm of the toad, *Bufo bufo japonicus*. *Gamete Res.* **6**, 343–52.