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Oocyte-Follicle Cell Gap Junctions in *Xenopus laevis* and the Effects of Gonadotropin on Their Permeability

Abstract. *Junctions between* Xenopus laevis *oocytes and follicle cells have been identified as gap junctions by the passage of microinjected fluorescent dye from oocytes to follicle cells. The opening or assembly of these junctions, or both, appears to be regulated by gonadotropins.*

The functional relation between the amphibian oocyte and the single layer of follicle cells that surrounds it is not well understood. Follicle cells have been implicated in a number of gonadotropin-regulated processes involved with amphibian oocyte growth and development. These include steroidogenesis (*1*), initiation of yolk protein (vitellogenin) uptake (*2*), and increases in amino acid uptake and protein synthesis in the oocyte (*3*). Ultrastructural examination of the ovary of *Xenopus* has shown that the follicle cells possess numerous macrovilli which project through the vitelline envelope and contact the oocyte surface, forming junctional complexes of an unidentified nature with the oocyte membrane (*4*). Recently we have observed, with the aid of lanthanum tracers, that there are small gap junctions between the follicle cell macrovilli and the oocyte in *Xenopus* (Fig. 1A). Gap junctions have also been identified in the mammalian ovary between granulosa cells and oocytes (*5*). In this report we present evidence that the junctional complexes between the amphibian oocyte and its follicle cells are gap junctions and that these junctional complexes may be hormonally regulated.

Gap junctions, through their structural modifications of the membranes of adjointed cells allow for cell to cell communication by the passage of ions and of molecules of low molecular weight (*6*). To determine whether the junctional complexes observed between the amphibian oocyte and its surrounding follicle cells are gap junctions, we isolated complete follicles (*4*) from *Xenopus* ovaries and injected approximately 50 nl of the fluorescent dye, 6-carboxyfluorescein through the follicular wall into the oocytes. Fluorescein and its derivatives pass freely through gap junctions, but do not readily permeate nonjunctional membranes (*6*). The injected dye was allowed to diffuse through the ooplasm for 1 to 2 hours. Smaller injection volumes or shorter diffusion times result only in lower levels of fluorescence in responding oocyte-follicle cell complexes. Two hours after injection the oocytes in their follicles were transferred to a solution of 1 m*M* phenylarsine oxide in a double-strength salt solution [OR 2 (*7*)]. Phenylarsine oxide, a sulfhydryl agent, stiffens the theca, the follicle cell layer, and the acellular vitelline envelope, while the hypertonic solution causes the oocyte to shrink slightly and separate from the theca and the follicle cell layer. This separation allows the two layers to be easily removed, one after the other, with watchmaker's forceps. Very often the vitelline envelope and the follicle cell layer are removed as a single unit. For our purposes this technique proved to be superior to the standard means for removing follicle cells which involves placing dissected oocytes in Ca^{2+}-Mg^{2+}-free or EDTA-containing media (*8*, *9*).

Follicle cell layers were examined from stage IV, V, and VI oocytes (*10*). Oocytes were taken from unstimulated animals and from animals that had been stimulated with 1000 I.U. of human chorionic gonadotropin (hCG) (*11*) 24 hours previously. Ten healthy females were stimulated with hCG. Nearly all follicle cell layers removed from the fluorescein-injected oocytes of the ten stimulated females showed fluorescence, indicative of the passage of a dye through a gap junction (Fig. 1, B and C). Fluorescence was observed in the follicle cell layers from oocytes of all stages examined. Normally not all of the follicle cells from the same oocyte fluoresced with the same intensity, although occasionally all were equally fluorescent. Neither uninjected oocytes enclosed in their intact follicles nor dissected oocytes enclosed only in follicle cells and exposed directly in fluorescein-containing medium cause fluorescence in follicle cells.

In some *Xenopus* ovaries, follicles containing two oocytes are present. Although these oocytes are enclosed within

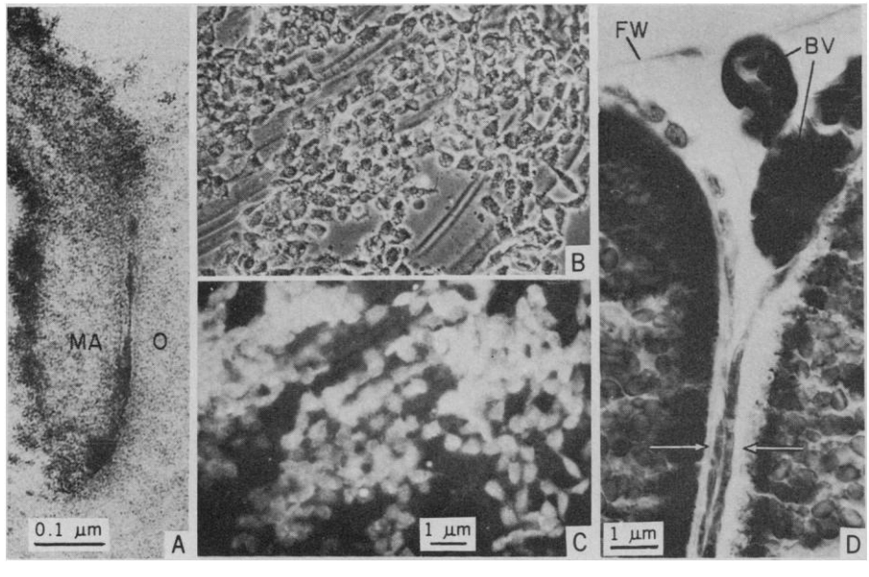

Fig. 1. (A) A small gap junction between a follicle cell macrovillus (*MA*) and an oocyte (*O*) demonstrated by means of a lanthanum tracer. (B) Phase-contrast and (C) fluorescent microscope images of the same field of follicle cells from an oocyte injected with 6-carboxyfluorescein from an hCG-stimulated animal. (D) Two oocytes enclosed in a common follicle (*FW*, follicular wall) but having individual follicle cell layers (arrows); *BV*, blood vessels in theca.

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a common theca, each oocyte has its own follicle cell layer (Fig. 1D). To show that the fluorescence observed in follicle cells is due to direct passage of the dye from the oocyte to the follicle cells and not due to leakage from the oocyte into the medium, we conducted ten experiments in which only one oocyte of the double follicle pair was injected with dye. Only the follicle cells from the injected oocytes showed fluorescence, leading to the conclusion that the dye was reaching the follicle cells directly from the oocytes and not from the intrafollicular space.

Unstimulated animals that did not receive hCG prior to dye injection had very low or no fluorescence in their follicle cells. This suggests that there were few, if any, functional gap junctions between these occytes and follicle cells. The only unstimulated animals that showed fluorescence in the follicle cells after injection of fluorescein into the oocytes were 5 out of 13 females that had been recently acquired from the supply house (South African Snake Farm, Fish Hoek). Follicle cells from oocytes from ten unstimulated animals that had been maintained in the laboratory for long periods of time did not fluoresce after injection of the dye into the oocyte.

To examine further the effects of hCG on intercellular communication between the follicle cells and the oocyte, we anesthetized seven recently acquired unstimulated animals by hypothermia and removed a portion of the ovary. After the incision was closed the animals were injected with 1000 I.U. of hCG. Oocytes from the excised ovary were injected with fluorescein and the follicle cells were examined for fluorescence. Twenty-four hours later, partial ovariectomies were again performed on the now hCG-stimulated animals, the oocytes injected with fluorescein, and the level of fluorescence in the follicle cells noted. In all seven animals the follicle cells showed little or no fluorescence. Twenty-four hours after hCG stimulation, however, both the number of follicle cells that fluoresced and the intensity of their fluorescence was greatly increased.

Apparently hCG, which seems to elicit effects similar to endogenous pituitary gonadotropins in *Xenopus*, stimulates the opening or assembly of gap junctions between the oocyte and its follicle cells. Variations in the levels of fluorescence in adjacent follicle cells suggest that the effect of hCG may be at the level of individual cells rather than affecting all cells of the follicular layer simultaneously. Such variations also imply that the follicle cells may not be completely interconnected by functional gap junctions as are mammalian granulosa cells (*5*).

If gonadotropin stimulation is responsible for the opening or assembling of functional gap junctions between the oocyte and follicle cells, the low levels of fluorescence found in follicle cells from a small number of unstimulated dye-injected oocytes is not surprising. The considerable variation observed in the rate of vitellogenin uptake in unstimulated (control) animals in vitro (*2*) suggests that natural hormonal levels are difficult to control under long-term laboratory conditions. This is supported by the observation that the unstimulated laboratory-maintained animals whose oocyte-follicle cell junctions either were not present or did not pass dye had a poor ovulatory response to hCG, whereas all newly acquired animals that showed some passage of dye prior to hCG stimulation and enhanced fluorescence after hCG stimulation had excellent ovulatory responses. It is possible that higher levels of endogenous gonadotropins may have been present in those animals which showed the presence of functional gap junctions without exogenous hormonal stimulation.

The functional significance of the gap junctions between the amphibian oocyte and its surrounding follicle cells is still unclear. In response to hCG stimulation the follicle cells secrete two clearly diffusible steroid hormones, one similar to progesterone that leads to oocyte maturation (*9*), and estrogen, which induces the synthesis and secretion of the yolk precursor protein vitellogenin by the liver (*12*). It has also been postulated that the follicle cells may be the source of a relatively nondiffusible factor which influences endocytotic uptake of vitellogenin by the oocyte in response to gonadotropic stimulation (*2*). Since it appears that follicle cells must be present on the surface of the oocyte for initiation of the in vitro uptake of vitellogenin, it is possible that this "initiator factor" is transmitted from the follicle cells to the oocyte directly by means of the gap junctions. In the mammalian ovary, the oocyte-granulosa cell gap junctions have been suggested as a means of maintaining meiotic arrest in the oocyte by transmitting an inhibitor from the follicle cells (*13*). There are as yet no data to support the theory of a similar function in amphibians.

It is significant not only that the gap junctions between the amphibian oocyte and its follicle cells may serve to transmit a hormonal stimulus, but also that the means of communication, a permeable gap junction, is hormonally regulated. It has been proposed that an important function of gap junctions is to allow the synchronized movement of small regulatory molecules between functionally related cells during growth and differentiation (*14*). More recently it was shown that rat granulosa cells pass hormonal stimuli by means of a second messenger through gap junctions formed in culture with mouse myocardial cells (*15*). Thus, in hormonally regulated developing systems such as the amphibian oocyte, gap junctions may allow the passage of secondary messages from the follicle cells to the oocyte in response to hormonal stimuli. Many aspects of *Xenopus* oocyte growth and differentiation. such as vitellogenin and amino acid uptake, protein synthesis, and maturation are under the control of hormones and can be induced in vivo and in vitro with hCG. Thus the ovary of *Xenopus* provides an excellent system in which the transmission of hormonal stimuli through naturally occurring junctions can be studied with respect to growth and differentiation.

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