Microfilament-Mediated Surface Change in Starfish Oocytes in Response to 1-Methyladenine: Implications for Identifying the Pathway and Receptor Sites for Maturation-inducing Hormones

THOMAS E. SCHROEDER
University of Washington, Friday Harbor Laboratories, Friday Harbor, Washington 98250

ABSTRACT Oocytes of the starfish *Pisaster ochraceus* exhibit an early response to 1-methyladenine (the maturation-inducing hormone), which is described for the first time. In this response ~6,500 spikelike surface projections, much larger than microvilli, emerge transiently from oocytes stripped of their follicle cells and then treated with the hormone in vitro. Each spike contains a prominent bundle of microfilaments, possibly composed of actin. The distribution of spikes when follicle cells are only partially removed and the morphological details of the normal junctional association between follicle cells and oocytes suggest that 1-methyladenine-sensitive sites (receptor sites) can be identified with the ~6,500 postjunctional specializations that are part of the oocyte surface. This finding in turn is employed to construct a set of hypotheses concerning the route that 1-methyladenine normally takes from the follicle cells to an oocyte during stimulation of maturation; it is postulated that, for each oocyte, 1-methyladenine is transported along ~6,500 thin follicle-cell processes, is transmitted across the junctional gaps of an equivalent number of junctions between follicle cells and an oocyte, and then interacts with the postjunctional sites where 1-methyladenine receptors are thought to be clustered. Comparative aspects of this mode of intercellular communication are discussed.

The regulation of reproduction in starfish (echinoderms of class Asteroidea) is achieved through hormone-mediated intercellular communications, as it is in other animals. Many important aspects of the route and actions of these hormones are still unknown. In the starfish ovary, oocytes are surrounded by a thin sheath of follicle cells. A jelly coat and vitelline layer intervene between these cell types, except where thin processes from the follicle cells penetrate them and form characteristic "desmosome-like" junctions with the oocyte surface (50). When spawning commences, oocyte maturation and ovulation are hormonally triggered and soon afterward massive numbers of oocytes without follicle cells are released into the seawater. These oocytes are full-grown but are still immature in the sense of having intact germinal vesicles. Maturation, which is irreversibly triggered in the ovary, proceeds quickly and soon converts the immature oocytes to fertilizable mature oocytes.

The maturation-inducing substance in starfish is 1-methyladenine (1-MA) (36, 37) and is produced by the follicle cells (29). It is released by them in response to a chain of events that includes secretion of a polypeptide hormone from the radial nerves (the "radial nerve factor") (30, 36, 37). 1-MA is thought to interact with receptors on the oocyte surface (15, 38, 43, 44), but intermediate steps from its origin in follicle cells to those receptors are unknown. How is 1-MA passed between the cells? How is it received and recognized? Are the sites of 1-MA release and reception localized or widely dispersed?

Fortunately for experimentation, maturation events in starfish can be triggered in vitro by treating isolated oocytes with exogenous 1-MA. Until now the first obvious sign of oocyte maturation has been the visible breaking-down of the germinal vesicle. In the present study, I will describe a much earlier response to 1-MA. In interpreting that response, I find the circumstantial basis for a set of hypotheses concerning the normal pathway of 1-MA from the follicle cell to the oocyte and the identity of the receptor sites for 1-MA on the oocyte surface.

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MATERIALS AND METHODS

Oocytes

Adult specimens of *Pisaster ochraceus* were collected intertidally from several sites around San Juan Archipelago, Washington from March through August of 1979 and 1980. They were stored in running seawater for varying periods until they were used in experiments; in general they did not spawn continuously under these conditions. In nature *Pisaster* spawns from late May to early June, the precise time probably being a function of several environmental factors. Oocytes with their investing follicle cells were generally obtained by severing an arm from a female, removing the two ovaries into a bowl of seawater, and mincing and shaking them with forceps. Oocytes surrounded by their follicle cells were then rinsed in several changes of seawater and kept at 11°C. Most experiments were conducted at this temperature, with spot observations of subsamples being checked with the light microscope at 21°C. Alternatively, some preparations were continuously observed by light microscopy at 11°C or 21°C, using a thermoelectric cooling stage.

Calcium-free Seawater

A balanced and buffered artificial seawater lacking calcium ions was prepared according to a formulation published elsewhere (9). It was chelated with EGTA and buffered to pH 8.1 with Tris. To remove follicle cells, oocytes were washed in five changes of this calcium-free seawater.

Removal of the Jelly Coat and Vitelline Layer

These layers were routinely and thoroughly removed by digestion in proteolytic enzymes after calcium-free seawater washes by procedures that will be described in detail elsewhere.[1] Evaluation by light and scanning electron microscopy confirmed that each preparation was uniformly denuded and that the response to 1-MA after this treatment was similar to controls.

1-MA

This maturaton-inducing substance (1-MA) was purchased from Sigma Chemical Co., St. Louis, Mo., and was stored frozen in a stock solution of 2×10^{-3} M in distilled water. When large numbers of synchronous oocytes were desired, 25 μl of the stock solution were added to a few thousand oocytes suspended in 5 ml of natural or artificial seawater. If a small culture was needed, a working solution of 2×10^{-5} M 1-MA was diluted from the stock into either natural or artificial seawater, whichever was appropriate for the oocytes. A drop of this was then combined with a drop of oocyte suspension on a microscope slide.

Fixation

Although many fixation protocols were explored, the following procedure proved most successful. For scanning electron microscopy, oocytes were added to a 1:9 mixture of 10% glutaraldehyde and the appropriate seawater for 1–2 h and postfixed in a 1:3 mixture of 4% osmium tetroxide and seawater for another hour. For transmission electron microscopy, oocytes were added to a 2:23 mixture of 25% glutaraldehyde and seawater for 1–2 h, rinsed in 1.25% sodium bicarbonate (pH 7.0) for ~5 min, and postfixed in 1% osmium tetroxide in 1.25% sodium bicarbonate for 1 h.

Negative Staining and Heavy Meromyosin

Surface spikes were stripped from the surfaces of denuded oocytes as has been described for microvilli from eggs (9). This involved settling a few oocytes onto a polylysine-treated, carbon-coated grid, shearing them with a jet of buffered salt solution, applying uranyl acetate, and drying. After such preparations were sheared open, heavy meromyosin was applied at a concentration of 10 mg/ml for 5 min. Six different batches of heavy meromyosin were used and minor aspects of the procedure were varied but the results were similar in all cases.

RESULTS

General Features of the Oocyte Preparations

When oocytes are shaken from a minced ovary into seawater, they emerge with follicle cell sheaths more or less intact (Fig. 1). An oocyte has a diameter of ~160 μm and the flagellated squamous follicle cells are separated from it by a distance of 10–25 μm (Figs. 1–3). The intervening space is filled with the jelly coat, which gradually swells in seawater and appears flocculent or lightly fibrous in the light microscope (Fig. 2). The vitelline layer is intimately associated with the oocyte and is ~0.5 μm thick (Figs. 4 and 5). Numerous microvilli ~0.15 μm wide and 0.4 μm long are embedded in this layer.

Follicle cells can be removed by several washes in the calcium-free seawater, but the other layers remain intact. The jelly coat and vitelline layer are completely removed by enzymatic digestion, which leaves the oocyte denuded down to the plasma membrane. It is important to note that 1-MA can be applied to oocytes with any or none of these extraneous layers, in either natural or artificial seawater, with perfectly comparable results, with only one exception: when a complete sheath of follicle cells is still present, maturation proceeds much less uniformly or synchronously than after they are removed. This conforms with previous findings reported by others (13, 53).

As early as March *Pisaster* oocytes are virtually full-grown and respond to 1-MA by undergoing germinal vesicle breakdown; that is, this important feature was constant throughout the period of these experiments. On the other hand, other aspects did vary with the reproductive season, in particular the appearance of spikes (see below). In addition, the number of follicle cells surrounding a freshly isolated oocyte varies irregularly with the batch and time of year. A full complement of follicle cells (Fig. 1) comprises 50–100 cells, based on scanning electron micrographs. In general, the number of adhering follicle cells early in the reproductive season tends to be greater than the number later in the season, as if the contact between follicle cells and oocytes weakens over this period. Nevertheless, follicle cells associated with a given oocyte will remain in place for an indefinite number of days if the complexes are kept in seawater after isolation. After 1-MA is applied to follicle cell–oocyte complexes from *Pisaster*, contractility or aggregation of follicle cells is not observed, contrary to reports for other species (10, 35, 36, 49).

Often ~5% of the oocytes in a batch exhibit ruptured germinal vesicles even before treatment with 1-MA, presumably as a result of some kind of trauma incurred through handling. These oocytes do not change with time and do not form polar bodies, so they should not be considered examples of so-called "spontaneous" maturation that occurs in some species (54).

Histology of the Follicle Cell–Oocyte Association

The space between a *Pisaster* oocyte and its follicle cells is traversed by a large number of thin, unbranching, radially arranged cytoplasmic processes (Figs. 2 and 3). Follicle cell processes emanate from cusps of cytoplasm on the undersides of follicle cells, pass through the jelly coat and vitelline layer, and form characteristic intercellular junctions with the oocyte surface (Fig. 4), as has been discovered independently in another starfish, *Patiria miniata*, quite recently (50). The follicle cell processes are ~0.15 μm in diameter and lack all major cytoplasmic organelles and inclusions. Cytoplasmic material in the processes is often amorphous but sometimes is distinctly microfilamentous. Each follicle cell process appears to form a single junction with the oocyte surface.

The follicle cell–oocyte junction (Fig. 4) is composed of a prejunctional component belonging to the follicle cell and a postjunctional component of the oocyte. Electron micrographs

[1] Schroeder, T. E., and S. A. Stricker. Manuscript in preparation.

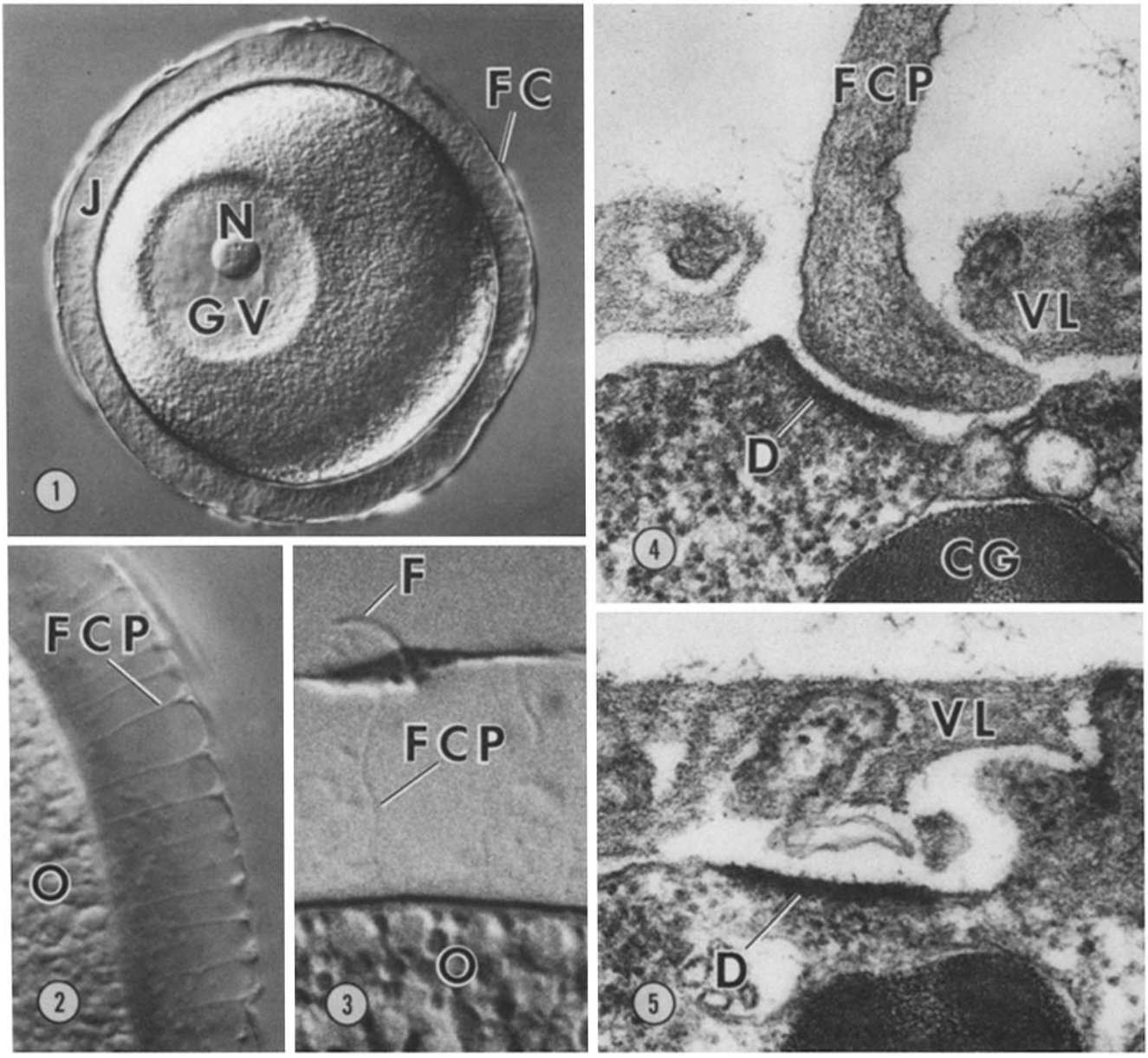


FIGURE 1 Light micrograph of a living starfish oocyte and its surrounding layer of very thin follicle cells (*FC*), as experimentally removed from the ovary. Follicle cells are present as a complete sheath early in the season and tend to be lost later in the season when oocytes are removed from an ovary for experiments. Naturally spawned oocytes are not associated with follicle cells. *GV*, germinal vesicle; *N*, nucleolus; *J*, jelly coat. Slightly compressed. Nomarski optics, × 350.

FIGURE 2 At higher magnification, numerous thin, 15-μm-long processes from the follicle cells (*FCP*) are seen to pass through the jelly coat and vitelline layer and to impinge upon the surface of the oocyte (*O*). Nomarski, × 1,500.

FIGURE 3 Additional details of a follicle cell, including its single flagellum (*F*) and a follicle cell process (*FCP*). *O*, oocyte. Unstained, 3-μm-thick Epon section. Nomarski, × 3,000.

FIGURE 4 Transmission electron micrograph of the follicle cell–oocyte junction of a full-grown immature oocyte. The follicle cell process (*FCP*) penetrates the jelly coat and vitelline layer (*VL*) and terminates in a slight swelling. A 20- to 35-nm-wide gap separates the follicle cell and the oocyte. A sub-plasma membrane density characterizes each side of the junction; the postjunctional density (*D*) belonging to the oocyte is more pronounced. *CG*, cortical granule. × 80,000.

FIGURE 5 The postjunctional density (*D*) of an immature oocyte persists after follicle cells are removed by washing in calcium-free seawater. *VL*, vitelline layer. × 80,000.

indicate that a junctional space or gap 20–35 nm wide separates the two plasma membranes and is bridged by a vaguely fibrillar material. The postjunctional component is a flat or slightly concave disk ~0.4 μm in diameter that occurs among the microvilli of the oocyte surface. The terminus of the follicle cell process is slightly expanded and contains no vesicles. Each junctional component exhibits a layer of dense material ~30 nm thick on the cytoplasmic side of each plasma membrane (Fig. 4). The density of the postjunctional component is slightly more pronounced. The cytoplasm immediately associated with

these dense specializations is indistinguishable from cytoplasm elsewhere. There is little evidence of organized microfilaments in the cortex of unstimulated oocytes.

Washing in calcium-free seawater dislodges follicle cells and their processes by disrupting follicle cell–oocyte junctions. After such treatment, the postjunctional specializations persist without obvious alteration (Fig. 5) as electron-dense patches in smooth places between microvilli. All features of these specializations are preserved even though the junctions themselves are broken, including remnants of the fibrous material that oridinarily bridges the junctional gap.

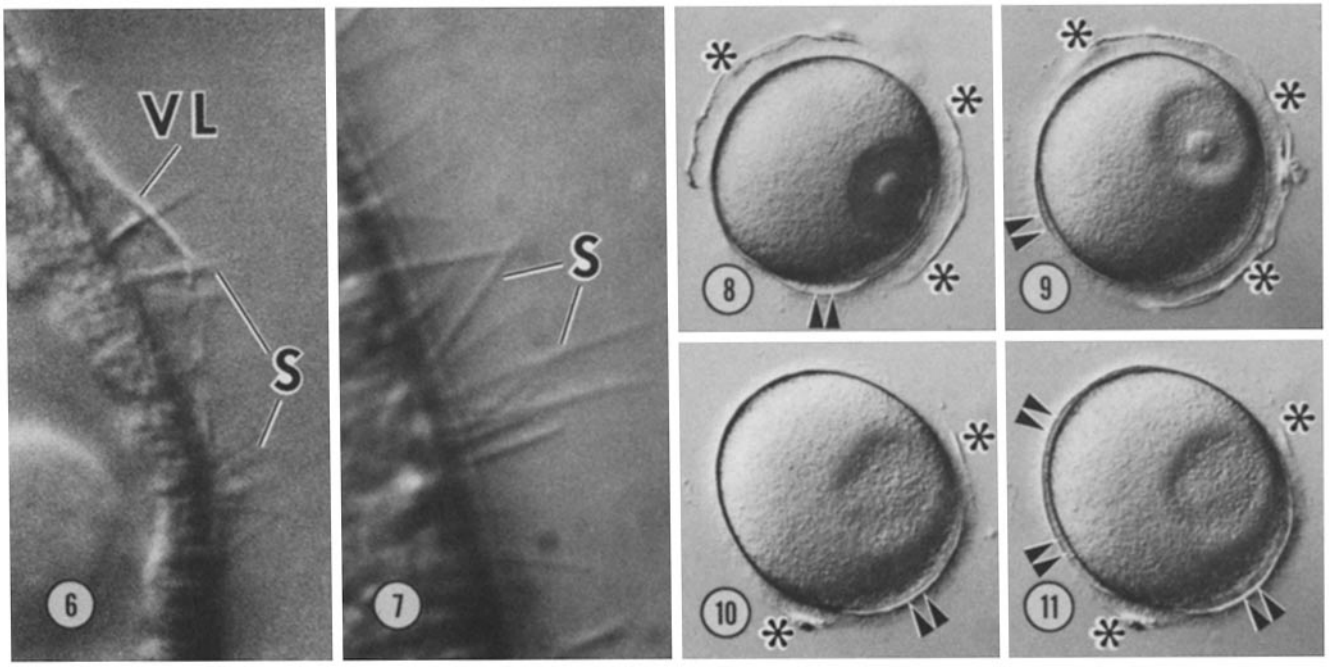
Spikes: An Early Response to 1-MA

When exogenous 1-MA (10^{-5} M) is applied to full-grown immature oocytes, the germinal vesicles break down ~60 min after treatment at 11°C (25 min at 21°C). Germinal vesicle breakdown is synchronous and consistent, regardless of any pretreatments for removing extraneous coats, except when complete sheaths of follicle cells are present, which impedes the action somewhat. Germinal vesicle breakdown can be triggered by 1-MA from March through August, even though the natural spawning season is late May to early June.

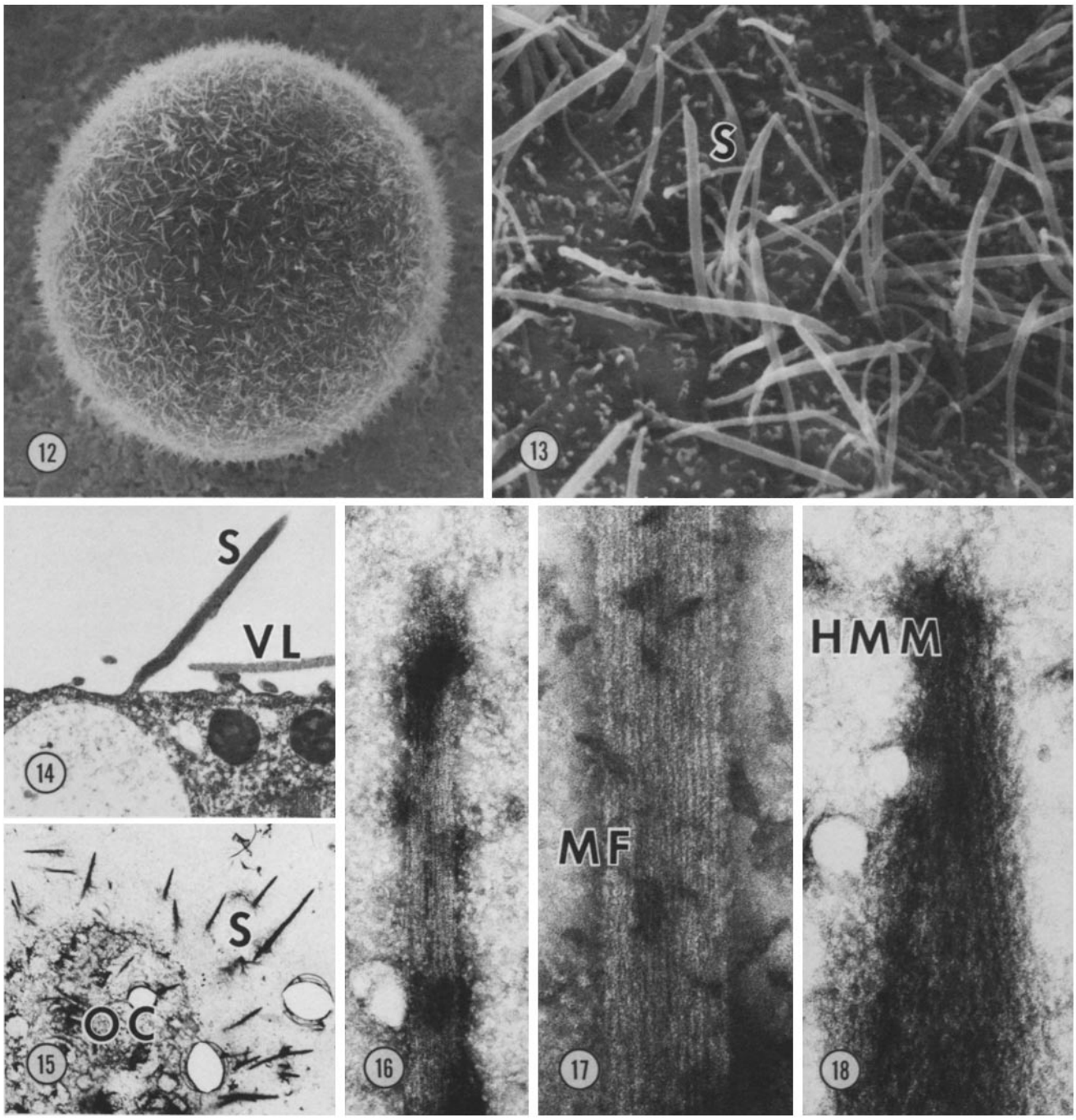
Oocytes obtained in late May through August exhibit an additional and striking early change that is not seen in earlier batches. Within a few minutes after application of 1-MA, numerous spikelike projections emerge from the oocyte surface except where it is covered by follicle cells. These spikes are easily observed in the Nomarski differential interference contrast microscope (Figs. 6 and 7) and cover the entire surface. They appear prominently in scanning electron micrographs (Figs. 12 and 13). Spikes first appear at ~5 min at 11°C (more quickly at higher temperatures) and elongate to 5–15 μm by 10 min; they remain as inflexible and immotile projections for many minutes and then disappear by ~30 min (20 min at 21°C). In all cases spikes appear and disappear before the germinal vesicle breaks down in response to 1-MA. Spikes have not previously been reported and represent an unprecedented response to 1-MA in terms of their character and rapidity of formation.

Within a responsive batch, oocytes form spikes equivalently regardless of the presence or absence of the jelly coat or vitelline layer and despite the various treatments used to remove these materials. They form in natural and artificial calcium-free seawater. Nevertheless, some curious seasonal differences between batches have been observed. In 1979, when 1-MA was applied to oocytes surrounded by vitelline layers, spikes formed and consistently lifted the vitelline layer (Figs. 8–11). In 1980, surface spikes usually penetrated the vitelline without lifting it (Fig. 7), or occasionally lifted it part way as well as penetrating it (Fig. 6). No explanation has yet been found for this difference.

A further aspect of the pattern of spike formation was observed in batches late in the 1979 season but never in 1980, presumably because of the different behavior of the vitelline layer. Oocytes shaken from an ovary were surrounded by only a few follicle cells, as if many had sloughed off in the process. When 1-MA was applied directly to these oocytes in seawater, spikes formed and the vitelline layer was lifted—but only where an oocyte was not obviously covered by a follicle cell (Figs. 8–11). When oocytes from the same batch were treated with calcium-free seawater to remove all follicle cells, spikes then formed over all parts of the surface. This behavior suggests that regions of oocytes that are intimately associated with



FIGURES 6–11 When oocytes are treated with 1-methyladenine (1-MA), thousands of cytoplasmic projections called "spikes" (*S*) emerge from the surface. They achieve their full length of 5–15 μm in ~10 min and then start to recede. The formation of spikes sometimes lifts the vitelline layer (*VL*) (Fig. 6); in other batches they penetrate it (Fig. 7). When a few follicle cells are present, as in Figs. 8–11, the vitelline layer lifts (double arrowheads) only in regions that are obviously devoid of follicle cells and never beneath them (asterisks). This indicates that the areas beneath follicle cells are not responsive to 1-MA, at least in terms of producing spikes. Figs. 10 and 11 illustrate the same oocyte at 2.0 and 5.5 min after 1-MA, respectively; notice that gradual lifting of the vitelline layer everywhere except beneath the follicle cells. Fig. 6, Nomarski, × 1,500. Fig. 7, × 2,000. Figs. 8–11, × 200.



FIGURES 12 AND 13 Scanning electron micrographs of spikes (*S*) produced by 1-MA. The spikes are 5–15 μm long and ~0.5 μm thick. They uniformly cover the entire surface of the oocyte. Because this preparation was enzymatically digested to remove the jelly coat and vitelline layer before 1-MA was applied, the surfaces seen here represent naked plasma membrane. Somewhat shrunken in preparation. Fig. 12, × 660. Fig. 13, × 6,600.

FIGURE 14 When thin-sectioned for electron microscopy, the interior or core of a spike (*S*) consists of a tight bundle of microfilaments that are not easily resolved. *VL*, vitelline layer. × 10,000.

FIGURE 15 To reveal the contents of spikes (*S*), this preparation has been attached to a polylysine- and carbon-coated grid, subjected to a shearing stream of salt solution, and then negatively stained. *OC*, remnant of oocyte cortex. Electron micrograph, × 1,200.

FIGURES 16 AND 17 Higher magnifications of negatively stained cores of spikes. In general these are too dense and thick to permit microfilaments to be resolved, except in especially thin spikes or near a tip. Individual microfilaments (*MF*) appear to be ~5 nm thick. Fig. 16, × 100,000. Fig. 17, × 200,000.

FIGURE 18 The core microfilaments of a spike after incubation in heavy meromyosin solution (*HMM*) for 3 min. Individual microfilaments even in this narrow portion near a tip are very difficult to discern but they give evidence of being "decorated" by the heavy meromyosin, indicating the possible presence of actin. × 200,000.

follicle cells are unresponsive to applied 1-MA, at least in terms of spike formation. This observation is a key one for the hypotheses advanced below.

Oocytes that have once formed spikes will not do so again. Further addition of 1-MA fails to elicit a second round of spikes, even if the oocytes are washed between treatments.

It is interesting to note that the small number of oocytes that lack germinal vesicles, apparently because of traumatic handling as mentioned above, respond to 1-MA with exaggerated spikes. In these oocytes spikes form particularly quickly, grow longer, and are slower to recede than spikes on normal oocytes. This may be interpreted to mean that the contents of the germinal vesicle alter the state of the cytoplasm or surface in its responsiveness to 1-MA.

The formation of spikes and the breakdown of the germinal vesicle exhibit similar dose-dependencies when the concentration of 1-MA is varied between 10^{-8} and 10^{-5} M. At 10^{-6} M or higher, both responses, spike formation and germinal vesicle breakdown, always occur. At 10^{-7} M or lower, neither response occurs. On the other hand, spike formation can be dissociated from germinal vesicle breakdown by pretreating oocytes with cytochalasin B for 30 s. Pretreating oocytes with 3.3 μg/ml cytochalasin B completely prevents the formation of spikes by 1-MA even though germinal vesicles break down normally. Very short spikes form in 0.33 μg/ml cytochalasin B. Spikes form normally in 0.033 μg/ml cytochalasin B and on all controls using dimethylsulfoxide, the solvent for cytochalasin B, up to a concentration of 0.33%.

Ultrastructure of Surface Spikes and Their Contents

Scanning electron micrographs (Figs. 12 and 13) reveal fine details of the pattern of spike formation, the simultaneous loss of microvilli, and the eventual disappearance of spikes, as will be described elsewhere.[1]

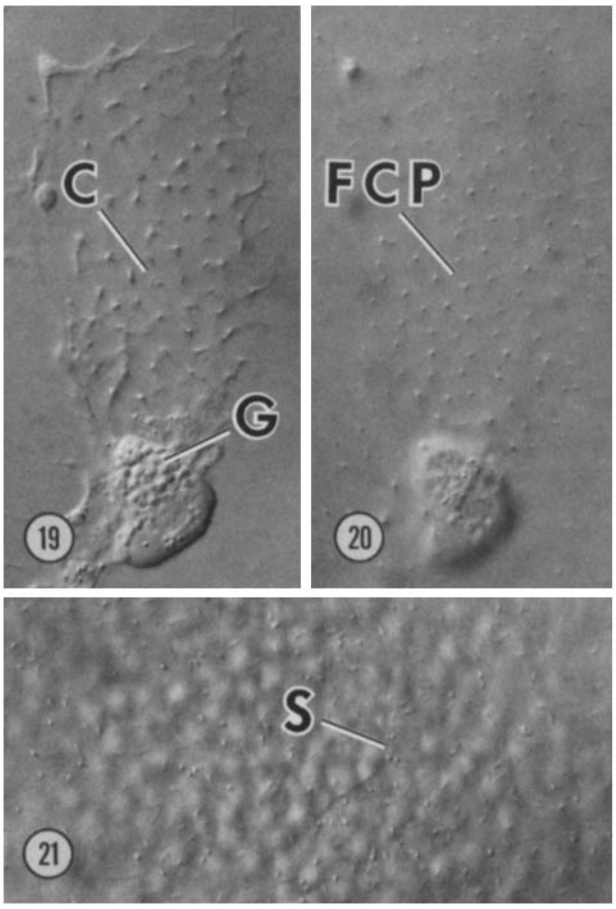
Fully formed spikes are 0.2- to 0.5-μm-wide cylinders, except at their tips where they taper abruptly (Fig. 13). Transmission electron micrographs of thin-sectioned spikes reveal prominent cores of electron-dense material (Fig. 14). These cores are very tight bundles up to 0.5 μm thick of microfilaments which are usually not separately resolved because of the degree of compaction. The presence of microfilaments is more readily appreciated in negative-stained preparations of isolated spikes, although even here the thickness of the bundles still interferes with their visibility. Figs. 15–18 are negative-stained specimens.

In negative-stained preparations, individual microfilaments can be seen clearly only at the tips of spikes (Fig. 16) or in a spike that is particularly narrow (Fig. 17). Individual microfilaments measure 4.5–6.0 nm in thickness, which is similar to that of actin.

The presence of actin in spike microfilaments can theoretically be confirmed with heavy meromyosin (9, 33), which should produce complexes with periodic "arrowheads." Although such "arrowheads" were not actually observed, a definite morphological alteration occurs when a core bundle of microfilaments is incubated in heavy meromyosin (Fig. 18). Relative to untreated controls (Fig. 17), core microfilaments appear to splay apart and a general cross-hatched pattern is visible, but fine details seem to be obscured by the thickness and extreme density of the structures. Different batches of heavy meromyosin produced the same effects. Thus, although it seems likely that core microfilaments of spikes contain actin, more positive proof and further characterization is clearly desirable.

The Numbers of Follicle Cell Processes, Junctions, and Spikes

The number of follicle cell processes per ooccyte—and of follicle cell-oocyte junctions per oocyte, as there is a one-to-one relationship—can be estimated from living cells by focusing the Nomarski microscope at the level of the follicle cells (Figs. 19 and 20). The lateral boundaries of these very thin cells are only vaguely seen, but individual nuclei, the perinuclear cytoplasm and its granules, flagella, and the large expanses of cytoplasmic cusps are easily seen. The follicle cell processes can be followed from their origins at these cusps down onto the oocyte surface at lower levels of focus. The concentration of follicle cell processes (number per unit area) can be determined from photomicrographs similar to Fig. 20; an ex-



FIGURES 19 AND 20 Surface views of a living follicle cell that is associated with its oocyte. A high plane of focus (Fig. 19) reveals the cell outline, intracellular granules near the nucleus (*G*), and numerous cusps (*C*) that correspond to the sites of origin of follicle cell processes. A slightly lower plane of focus (Fig. 20) shows that the follicle cell processes (*FCP*) can be traced right down to the oocyte surface. These structures can be counted in such small sample areas and the total number per oocyte can be determined by extrapolation. Nomarski, × 1,000.

FIGURE 21 Surface view of a naked oocyte treated with 1-MA. The numerous spikes (*S*) are seen end-on. The number of spikes is similar to the number of follicle cell processes shown in Fig. 20. Nomarski, × 1,000.

trapolation from such a photographed sample area to the area of the appropriate sphere (diameter of 190 μm at the level of the cusps) yields a fairly good estimate of the number of follicle cell processes per oocyte.

Spikes can be counted in either of two ways: from scanning electron micrographs of enzyme-denuded oocytes or from high-magnification Nomarski photomicrographs taken at a focal level that reveals the spikes end-on (Fig. 21). By either counting method, the number of spikes is determined in a sample area and extrapolated to the full surface area of the oocyte. The diameter of living oocytes is ~160 μm, so this value is used for the light microscope method. However, a compensation must be made for the shrinkage that occurs in preparations for scanning electron microscopy, so the oocyte diameter used for these extrapolations is determined for each oocyte from a low-magnification micrograph of the ooctye being studied, as in Fig. 12.

Table I summarizes the quantitative results of counting follicle cell processes and spikes. In summary, there are roughly as many spikes as follicle cell processes, considering that: (*a*) each set of determinations is from a different batch of oocytes; (*b*) variation between individual oocytes is quite high; and (*c*) each method of counting contains its own unique error. Moreover, because there is one follicle cell–oocyte junction associated with each follicle cell process, these results indicate that there are ~6,500 junctions per oocyte before 1-MA treatment and ~6,500 spikes after 1-MA treatment. Implications of this relationship are developed in the following sections.

DISCUSSION

The General Significance of Spikes and Their Relation to Maturation

Surface spikes that form on starfish oocytes treated with 1-MA represent the earliest recorded morphological response by any oocytes to a maturation-inducing hormone. However, because they form only under a restricted set of condtions, their ultimate significance must be interpreted with care. On one hand, considerable importance can be attached to the fact that they form at all; on the other, it is certain that spikes as such are not necessary prerequisites for oocyte maturation, at least as judged by breakdown of the germinal vesicle. For instance, germinal vesicle breakdown in *Pisaster* oocytes can be induced from March to May, before spikes are ever seen; later, when spikes are a regular feature of the 1-MA response, treatment with cytochalasin B inhibits their appearances without interfering with germinal vesicle breakdown.

Thus germinal vesicle breakdown and spike formation are two responses to 1-MA that can be uncoupled. Nevertheless, it has also been shown that they share the same threshold concentration of 1-MA, so they may also share a common kind of surface receptor for the hormone. The two responses may represent separate intracellular events that are linked only by a single hormone-receptor interaction.

Each 1-MA-triggered spike contains a prominent bundle of microfilaments, whereas the cortex of untreated oocytes lacks any obvious precursor microfilaments. These facts suggest that a rapid mobilization of microfilament material into bundles is one of the secondary effects of 1-MA in *Pisaster* oocytes and that this response is causally responsible for erecting the spikes. It is likely that the core microfilaments of spikes contain actin, based on (*a*) the dimensions of the microfilaments, (*b*) their behavior after cytochalasin treatment, and (*c*) the morphological alteration brought about by heavy meromyosin. However, the absence of discrete "arrowheads" in heavy meromyosin–treated bundles argues that the identification of actin is not yet definitive.

In addition to spikes, other early responses to 1-MA in various starfish oocytes include rapid increases in cytosol calcium (17, 45), protein kinase activity (23, 24), and sulfhydryl-containing protein (40). Also, mechanical "stiffness" (46, 47) and amino acid uptake (32) both decrease and there are transient changes in membrane electrical properties (16). It is still too early in any of these studies to know whether or how any of these changes may be connected with the microfilament-mediated formation of surface spikes or even with meiotic maturation itself.

More profoundly, it remains unknown precisely what surface and cortical changes are elicited by 1-MA during normal spawning. Because spikes form only at certain times of the year and are produced by adding exogenous 1-MA to oocytes lacking follicle cells, it is reasonable to assume that spike formation represents a hypertrophy or exaggeration of normal oocyte behavior. It has yet to be determined what cortical changes akin to bundling of microfilaments are triggered by endogenous 1-MA and whether such effects have any bearing on an oocyte's fertilizability or subsequent development.

Considering surface changes as distinct from cortical changes, present results indicate that microvilli on *Pisaster* oocytes are replaced by spikes, perhaps by being stretched out passively. In other species, even though spikes as such have not been reported, microvilli are also said to shorten after 1-MA treatment (10, 31), a change that might sufficiently explain the loosening of the vitelline layer that is occasionally observed (11). In this connection, it may be relevant to recall that spikes can only be elicited once, as if the surface and/or cortex is irreversibly altered so that it cannot again respond to a second exposure to 1-MA. It is plausible that spikes or their normal counterpart have an important role in dispersing or inactivating 1-MA receptors by altering the oocyte surface.

TABLE I

Number of Follicle Cell Processes vs. Spikes per Oocyte

Structure/oocyte	Mean ±SD	Number of oocytes counted	Method of counting
Follicle cell processes	6,240 ± 1,363	11	LM*
Spikes	8,732 ± 2,908	7	LM‡
Spikes	5,290 ± 2,501	10	SEM§

* Counts based on light micrographs such as Fig. 20.
‡ Counts based on light micrographs such as Fig. 21.
§ Counts based on scanning electron micrographs.

Relationship between Follicle Cell Processes, Junctions, and Spikes

In ooctyes that regularly form spikes, it has been shown that spikes appear only over those portions of the oocyte surface lacking follicle cells, their processes, and (presumably) any intact follicle cell–oocyte junctions. That is, wherever junctions are intact, spikes are either inhibited or the receptive area for 1-MA is "masked." Furthermore, the rough numerical equivalence between follicle cell processes and spikes on an oocyte suggests, but does not in itself prove, that there is a one-to-one relationship between these diverse structures.

More precisely stated, there is circumstantial evidence that a spike may arise from a postjunctional specialization when it is stimulated by 1-MA. Unfortunately, attempts to confirm or refute this interesting possibility by noting the ultrastructural relation of postjunctional densities to spikes as they emerge have been frustrated because all signs of the densities seem to vanish soon after 1-MA application, even before spikes arise. Thus, the possibility that postjunctional specializations represent the sites of spike emergence is not resolved.

A corollary of this possibility, which is of course equally conjectural, is that the postjunctional specializations represent the sites where 1-MA receptors are located on the oocyte surface. Accordingly, spikes may arise in the immediate vicinity of a localized interaction between 1-MA and a cluster of its receptors. Even as a conjecture, this notion raises important issues that should be explored in depth.

Deeper Implications: "Synaptic" Communication between Follicle Cells and Oocytes?

The mode of 1-MA-mediated communication between follicle cells and oocytes has not yet been discovered, although it is well established for starfish that (*a*) 1-MA is the maturation-inducing substance, (*b*) 1-MA is produced and released by follicle cells, and (*c*) 1-MA receptors are situated on the surface rather than in the cytoplasm. What is lacking is any scheme that explicitly defines the pathway taken by 1-MA from follicle cells to oocytes and specifies the exact location of the receptors. The present morphological findings demonstrate a distinctive histological association between follicle cells and oocytes, confirming very recent discoveries (50), and appear to offer reasonable explanations for these problems.

Regardless of the ultimate meaning that is attached to spikes themselves, there is merit in pursuing the possible consequences of their presence and the other morphological evidence as they may relate to the natural route of 1-MA in the starfish ovary. This problematic topic is the specific subject of the following hypothetical scheme, illustrated diagrammatically in Fig. 22. Its aim is to synthesize the above-mentioned hormonal and histological interactions between follicle cells and oocytes as they participate in the control of oocyte maturation. It departs from the conventional view that follicle cell processes and follicle cell–oocyte junctions subserve the physical stabilization and metabolic support of the oocyte (19, 27, 52, 58).

I suggest that 1-MA (*a*) is transported intracellularly along follicle cell processes, (*b*) is transmitted extracellularly across the narrow junctional gaps of follicle cell–oocyte junctions when the follicle cells are appropriately stimulated, and (*c*) interacts specifically with receptors clustered at the postjunctional specializations on the occyte surface. This postulated pathway for 1-MA resembles the communicative pathway by neurotransmitters across neuronal synapses and neuromuscular junctions and it predicts where the effective endogenous 1-MA is normally located and where it binds. As in other instances of communication between cells based upon morphological and chemical specializations, transmission of maturation-inducing hormone across discrete intercellular junctions appears to offer advantages over a more diffuse system of hormonal control of oocyte maturation by follicle cells.

The scheme is hypothetical. Certain secondary details of the scheme are particularly uncertain but point to pitfalls that might be encountered in attempting to test the hypotheses by experiment. For example, follicle cell–oocyte junctions may be "leak-proof" with respect to endogenous, transmitted 1-MA so long as they remain intact. This is implied by the observation that spikes never form in response to exogenous 1-MA when a junction is still intact; this situation may mean that 1-MA cannot leak in from the outside to stimulate the postjunctional receptor sites when it is "masked" by an adjacent follicle cell process and, conversely, it might follow that endogenous 1-MA would encounter equal difficulties leaking out. Leak-proof junctions would tend to exclude 1-MA from the general extracellular spaces of the ovary during natural hormone release. Eventually, however, disruption of follicle cell–oocyte junctions is a prerequisite for ovulation, so at a certain stage 1-MA might begin to appear in the extracellular spaces as a result of continued secretion even after an oocyte uncouples from its follicle cells. This possibility shows the problems in interpreting experiments that seek to test the validity of the above postulated 1-MA pathway by determining the localization of 1-MA alone.

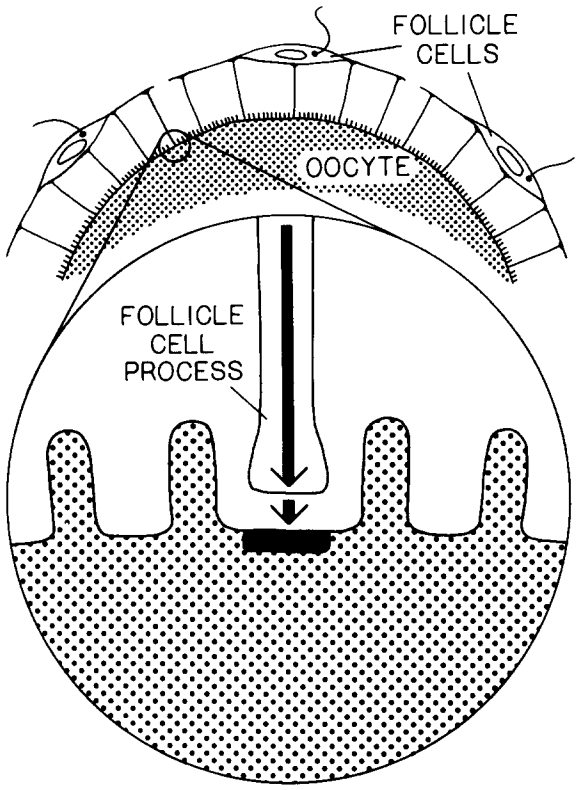


FIGURE 22 Diagrammatic representation of the hypothetical pathway (arrows) and receptor structure (dense bar) for the maturation-inducing "hormone" of starfish, 1-MA. 1-MA is (*a*) produced in the body of the follicle cell, (*b*) transported centripetally within the follicle cell process, and (*c*) transmitted across the narrow junctional space separating follicle cell and oocyte, where it is finally recognized by receptor molecules associated with the postjunctional complex on the oocyte surface. This model may be applied to the communication between follicle cells and oocytes in all vertebrates, including man.

Comparisons with Follicle Cell–Oocyte Interactions in Vertebrates

Although the present hypotheses have arisen from observations of starfish oocytes, it has not escaped notice that they may be equally relevant to other organisms in providing a needed

conceptual framework for understanding the control of maturation.

There are many analogies between maturation events in starfish and vertebrates. It is known that in all classes of vertebrates, except perhaps reptiles, which have not been extensively studied (4, 7), the histological association between follicle cells and oocytes resembles that in starfish and some other invertebrates (2). Thin processes from the follicle cells penetrate the extraneous layers of the oocyte and form intimate junctions with it. In amphibians it has been established that progesterone is the maturation-inducing substance, that it is produced and released by follicle cells, and that it acts on the oocyte surface (5, 12, 22, 48, 51, 56). The persistent attachment of follicle cells to oocytes impedes the action of exogenous progesterone in stimulating maturation in vitro (12, 34, 39, 41, 62), yet the response to progesterone becomes much more uniform and synchronous when the follicle cells have been removed. All of these details are reminiscent of the situation in starfish and similarly suggest that follicle cell–oocyte junctions in vertebrates may likewise represent leak-proof pathways for efficient progesterone transmission. The same corollary of this idea that was mentioned in connection with starfish—that follicle cell–oocyte junctions correspond to the sites of progesterone reception—is supported by the findings that the animal hemisphere of amphibian oocytes is simultaneously richer in junctions (6) and more sensitive to locally applied progesterone than the vegetal hemisphere (12).

Understanding the full functional role of follicle cell processes and junctions in oocyte maturation of vertebrates is complicated by the presence of gap junctions (1, 3, 8, 18, 21, 25, 59, 61) and of complex hormonal interactions dictated by specializations in reproduction that are not shared by starfish. Although the functions of the gap junctions are poorly understood, the interpretation of many mammalian studies is that they mediate an inhibitory influence upon the oocyte (14, 20, 26, 28, 35, 36, 42, 57, 58, 60), thereby supposedly restraining the resumption of meiosis. The present study does not challenge that interpretation, except to note that much of it relies upon so-called "spontaneous" maturation of oocytes, which occurs when follicle cells are removed and which in starfish is likely to be an experimental artifact (55). It is noteworthy that the literature on reproduction in mammals does not consider an alternate mechanism, whereby follicle cells actually exert a positive stimulatory control over oocyte maturation, similar to the mechanism proposed here (Fig. 22).

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