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The secretion of proteins *in vitro* from *Xenopus* oocytes and their accessory cells: a biochemical and morphological study

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SUMMARY

Protein secretion by *Xenopus laevis* oocytes and their surrounding follicular cells *in vitro* has been investigated using two-dimensional gel electrophoresis. Viable oocytes, devoid of follicle layers, were prepared by treatment with collagenase; they retain in full their capacity to synthesize, sequester and export secretory proteins following microinjection with heterologous messenger RNA. Both RNA-injected and normal cells export a large number of endogenous oocyte proteins and, as with heterologous secretory translation products, these proteins are found within the oocyte in a vesicle fraction. Electron microscopy indicates that secretion involves exocytotic release of cortical vesicle contents. The follicular cells themselves also seem to contribute a number of proteins to the incubation medium surrounding isolated oocytes, but the presence of follicle layers is not required for the export of endogenous oocyte proteins.

INTRODUCTION

The oocyte of *Xenopus laevis* has become a well-established system for the translation of microinjected mRNA's, offering the advantages of an '*in vitro*' environment in contrast to more traditional cell-free translation systems. Recent studies have demonstrated that the translation products of non-secretory mRNA's are retained in the cytoplasm of the cell whilst those of secretory message become compartmentalized within vesicles (Zehavi-Willner & Lane, 1977). Entry into the vesicle fraction appears to occur during synthesis of the nascent polypeptide chain (Lane, Shannon & Craig, 1979), failure to do so often resulting in proteolytic degradation of the secretory products. Proteins

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sequestered in this fashion undergo modification and are subsequently secreted by the oocyte (Colman & Morser, 1979). The possession of such a mechanism by *Xenopus* oocytes raises several questions. By what mechanism does the oocyte discriminate between secretory and non-secretory translation products? What role does secondary modification of secretory proteins play in the export process? What is the significance of the secretory system for the oocyte? We have recently undertaken a series of studies in an attempt to answer these questions. Thus, the export is neither cell-type-, species- nor even phylum-specific yet is highly selective (Lane *et al.*, in preparation). Further, the decisive event in this pathway is the entry of the newly synthesized polypeptides into the lumen of the endoplasmic reticulum rather than protein modification; later events in the release of sequestered, heterologous secretory proteins require the participation of microtubules (Colman *et al.*, in preparation).

In this paper, we report some studies directed towards elucidating the significance of the secretory pathway for the oocyte. Earlier work has suggested that, in addition to heterologous secretory proteins, oocytes or their surrounding follicle and theca cells secrete a number of endogenous proteins when cultured *in vitro* (Colman & Morser, 1979).

It is possible that all these endogenous frog proteins are secreted by the follicle layers; moreover the intimate association of these layers with the oocyte could prove essential for the secretion of heterologous proteins in response to secretory-message injection.

In the present paper we have resolved these possibilities using one- and two-dimensional gel electrophoresis to compare secretion of isolated oocytes in the presence and absence of their associated follicular layers. We have found that protein export is not a phenomenon elicited solely by the microinjection of the appropriate messenger RNA, nor is it dependant on the presence of follicle and theca cells. The normal oocyte, devoid of surrounding cells, is also active in the secretion of a large number of proteins.

MATERIALS AND METHODS

Oocyte incubation

Oocytes were injected with messenger RNA as described previously (Lane, Marbaix & Gurdon, 1971) and cultured in modified Barth X medium (Ford & Gurdon, 1977) containing the following additional antibiotics: Penicillin 100 units/ml, Streptomycin 100 units/ml, Gentamycin 70 μg/ml and Mycostatin 20 units/ml. Injected oocytes were left overnight to allow recruitment of message (Berridge & Lane, 1976) and healing of the micropipette wound. The cells were then labelled with [^{35}S]methionine (Radiochemicals Ltd, Amersham, Bucks., U.K.) using up to 600 μCi at 6 mCi/ml per 40 oocytes. Batches of 10–40 oocytes were incubated in label for 24–48 h and only healthy batches were processed.

Removal of follicle cells

Oocytes were initially defolliculated with collagenase (Sigma Biochemicals) using a concentration of 2 mg/ml in 0·1 M-phosphate buffer pH 7·5. Comparisons with untreated oocytes showed that this procedure often resulted in low levels of amino-acid incorporation and so an alternative method was devised. This involved overnight incubation at 20 °C in collagenase (4 mg/ml) dissolved in modified Barth X buffer, followed by manual removal of any remnants of the thecal layer. A further 4 h incubation in fresh collagenase solution, accompanied by periodic swirling, ensured the removal of the follicle cell layer. Most batches of oocytes treated in this way remained healthy for days or even weeks.

Oocyte fractionation

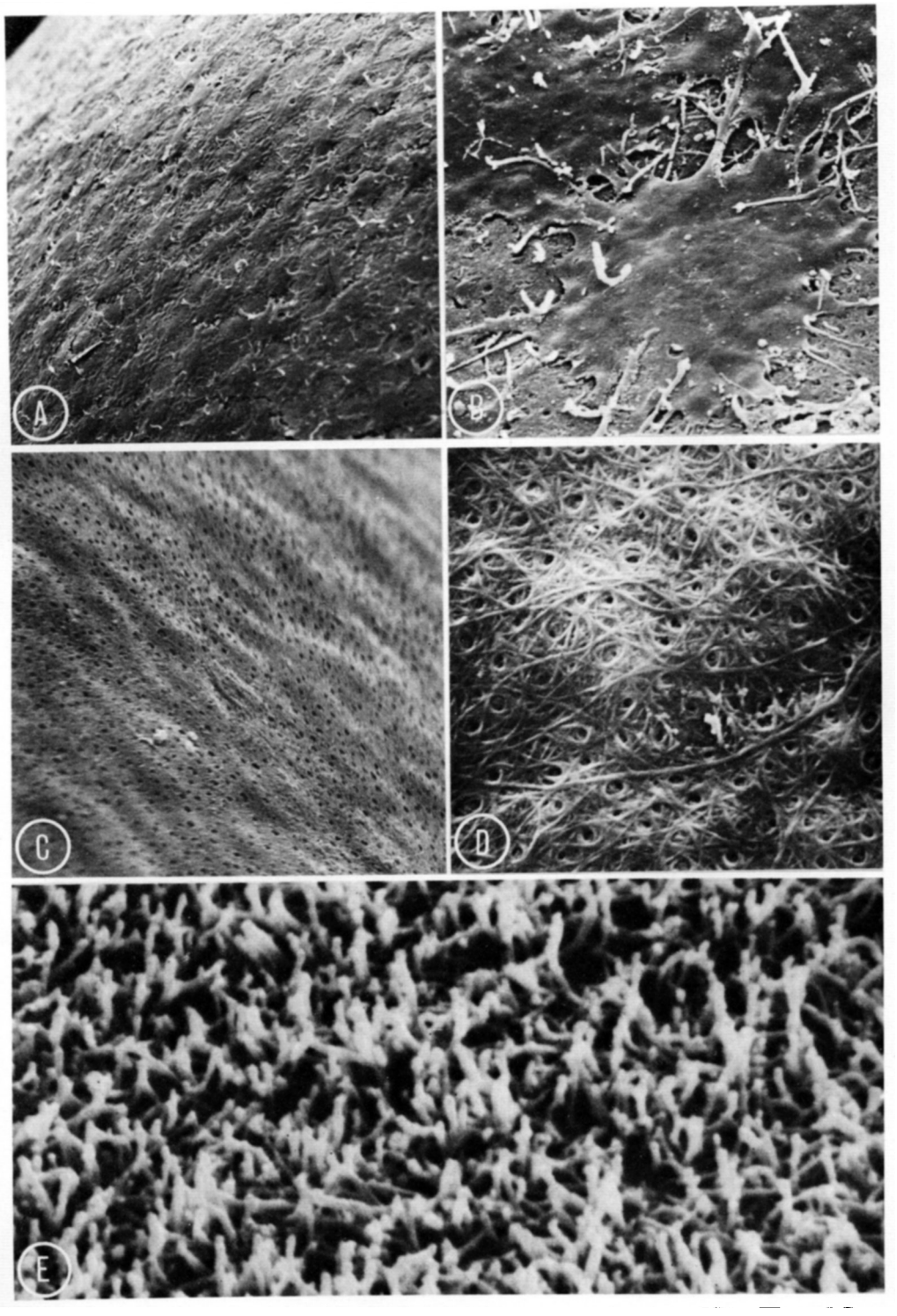
After incubation, the surrounding incubation medium was removed and frozen whilst the oocytes were fractionated into supernatant (cytosol) vesicle enriched, pellet fraction (DSI) and yolk pellet as described previously (Lane *et al.* 1979), except that sucrose (10% w/v) and additional KCL (150 mM) were present in the homogenization buffer. Acid-insoluble radioactivity in each fraction was determined by the method of Mans & Novelli (1961).

Electrophoretic analysis

One-dimensional electrophoresis on 12·5% polyacrylamide gels was performed essentially as described by Laemmli (1970) and Maizel (1971). Samples were prepared for electrophoresis by the method of Knowland (1974). Two-dimensional electrophoresis was carried out by the method of O'Farrell (1975) as modified by Ballantine, Woodland & Sturgess (1979). Second dimension electrophoresis was performed on 10% polyacrylamide gels. After electrophoresis, gels were fixed, dried and set up for autoradiography. Two-dimensional gels were fluorographed as described by Bonner & Laskey (1974) and Laskey & Mills (1975).

Electron microscopy (EM) of oocytes

Oocytes were fixed in half-strength Karnovsky's fixative (Karnovsky, 1965) in 0·1 M-sodium cacodylate buffer for 2 h, washed in buffer before post-fixing in 1% osmium tetroxide in 0·1 M-cacodylate buffer and dehydration in graded ethanols to absolute ethanol. Specimens for transmission EM were then embedded in araldite, gold sections taken, and stained with lead citrate and uranyl acetate before examination in a Phillips 301 electron microscope. Specimens for SEM were taken through graded concentrations of Freon 113 in ethanol to 100%, critical-point dried, gold sputter coated and examined in a Coates and Welter field emission SEM.


A
B
C
D
E

RESULTS

Isolation of follicle-free oocytes

The amphibian ovarian follicle is a complex structure consisting of a variety of cellular layers (Wischnitzer, 1963; Smith & Ecker, 1969; Wallace & Dumont, 1968). Each oocyte is surrounded by a tightly held, single layer of follicle cells intimately attached through the vitelline membrane to the underlying oocyte; a middle, thecal layer of connective tissue containing fibroblasts and elements of a capillary network; and an outermost surface layer of squamous epithelial cells.

Manual dissection of these layers from oocytes is a laborious procedure that often fails to remove the innermost layer of follicle cells adhering to the oocyte vitelline membrane (Schultz, 1967; Smith, Ecker & Subtelny, 1968; Jared & Wallace, 1969; Wallace, Jared & Dumont 1973; Wallace & Misulovin, 1978). It is therefore unsuitable for studies requiring individual follicle-free oocytes. Removal of the vitelline membrane ensures that all remaining follicle cells are also removed (see Fig. 1) but the resulting oocyte is too delicate for studies on the secretion of heterologous proteins.

To circumvent this problem, a variety of more drastic treatments have been adopted including EDTA treatment (Masui, 1967; Wallace *et al.*, 1973; Schatz & Ziegler, 1979), pronase digestion (Smith & Ecker, 1969; O'Connor, Robinson & Smith, 1977), collagenase digestion (Masui, 1967; Merriam, 1972; Eppig & Dumont, 1976; Eppig & Steckman, 1976) and exposure to papain with cysteine-HCl (Hallberg & Smith, 1976). We have found that overnight digestion with collagenase removes all follicle cells from the vitelline membrane as assessed by scanning electron microscopy (see Fig. 1). Initial attempts at collagenase treatment in phosphate buffer frequently resulted in deterioration of the oocytes as assessed by amino acid incorporation (up to 90% reduction in incorporation being observed). We have therefore adopted a procedure using modified Barth X buffer and oocytes treated in this way are as active in protein synthesis as untreated control cells, remaining healthy for days or even weeks.

The role of follicle cells in secretion of heterologous proteins

We have examined the contribution of follicle cells to oocyte secretion by comparing the response of collagenase-treated and normal oocytes to injection of a variety of messenger RNA's. Figure 2 shows the results obtained after

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Fig. 1. (A, B): Low (×750) and medium power (×3900) SEMs to show the appearance of the inner follicle cell layer after manual defolliculation.
(C, D): The same batch of oocytes after the second collagenase treatment (see Materials and Methods) to show the interwoven strands of the vitelline membrane fibres.
(E): After manual removal of the vitelline membrane. The surface of the oocyte itself is thrown up into microvilli. (×9600).

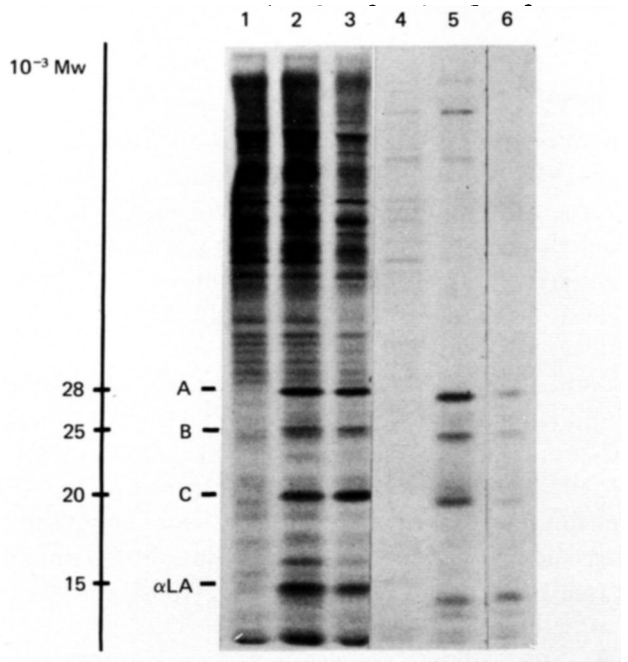


Fig. 2. Figure 2 shows the sequestration and export of endogenous and heterologous proteins in normal and follicle-free oocytes. Normal and collagenase-treated oocytes were injected with guinea-pig mammary gland RNA and, after a 20 h wound-healing period, were labelled with [^{35}S]methionine for a further 20 h. Cell fractions from RNA-injected and control oocytes were analysed on a 12·5% SDS-polyacrylamide gel. Tracks 1–3 show the vesicle fractions from uninjected oocytes, RNA-injected normal oocytes and follicle-free RNA-injected oocytes respectively; tracks 4–6 show incubation media from the same series of oocytes.

injection of guinea-pig mammary gland poly A$^+$-RNA. ^{35}S-labelled proteins were resolved by SDS-polyacrylamide electrophoresis and visualized by autoradiography. As previously reported (Lane *et al.* 1979) the predominant products from both cell-free and oocyte translation of this RNA fraction comprise caseins A, B and C and α-lactalbumin. These four products are found in the incubation medium of both defolliculated and normal oocytes (tracks 5 and 6 respectively). Removal of follicle layers does not therefore appear to impair the ability of oocytes to secrete heterologous proteins synthesized in response to microinjected mRNA.

Two-dimensional gel electrophoresis (O'Farrell, 1975) confirms these observations. Thus panels 1A and 1B of Fig. 3 show that the export of caseins A and B (identified from the known molecular weight of the immunoprecipitable oocyte casein products; Lane *et al.*, 1979) from normal and defolliculated oocytes. Casein C (21000 daltons) and α-lactalbumin (15000 daltons) migrate at the front of the second dimension gel and cannot therefore be detected. Caseins A and B (marked m1 and m2) show heterogeneity in charge and since this result is both highly reproducible and confined to these two polypeptides (and not to

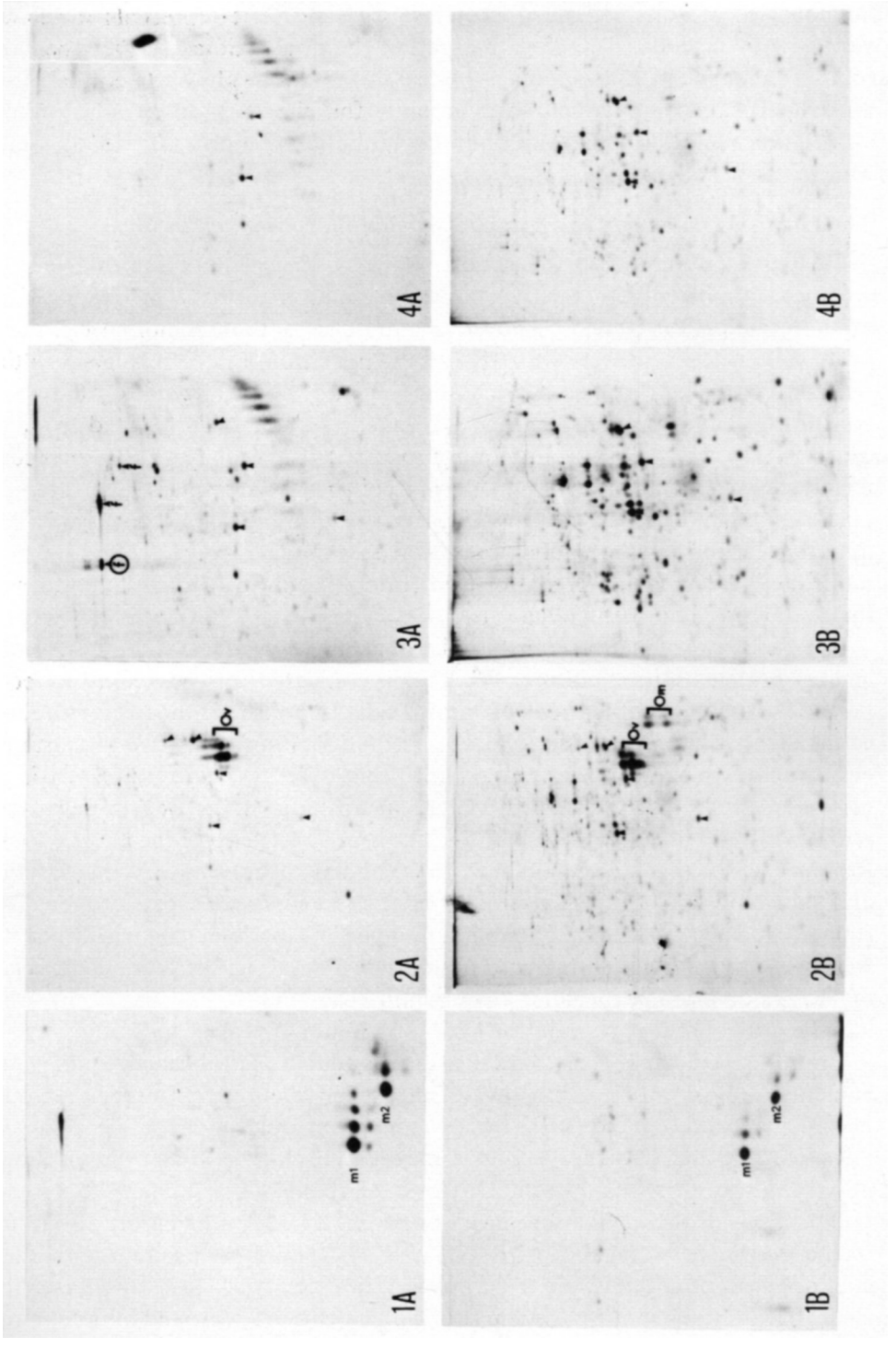
any oocyte- or follicle-derived proteins) we suggest it represents a genuine variation in secondary modification rather than an artifact of the isoelectric focusing procedure. Indeed guinea-pig caseins are both glycosylated and phosphorylated, and the latter could account for charge modification without significant alteration in electrophoretic mobility (Craig *et al.*, 1976; Craig, McIlreavy & Hall, 1978).

The origins of the minor polypeptides found in the incubation medium

Further examination of the results shown in Fig. 2 indicates that oocytes secrete a number of other polypeptides in addition to those programmed by microinjected RNA (tracks 5 and 6). The presence of these minor species could be explained in several ways. Firstly, they may be translation products resulting from less abundant guinea-pig mammary gland RNAs. Secondly, they may constitute oocyte proteins that have 'leaked' out of microinjected samples. Thirdly, they may result from damage incurred to the oocyte (and/or incompletely removed follicular layers) during collagenase treatment. All three explanations may be ruled out on the basis of the results shown in Fig. 2. The minor polypeptides are found in the incubation medium surrounding both uninjected (track 4) and RNA-injected (track 6) oocytes. In neither case were the samples treated with collagenase.

One further explanation remains: the oocyte may secrete a number of endogenous proteins during culture. We have investigated this possibility by comparing polypeptide populations of various cell fractions from oocytes with those of the incubation media, using the high resolution afforded by two-dimensional electrophoresis. Previous work has shown that a pellet fraction (DSI) enriched for vesicles may be obtained by detergent-washing of the oocyte pellet that remains after removal of aqueous supernatant proteins (Lane *et al.*, 1979). Proteins destined for secretion may be expected to be found in this fraction and panels 2–4 of Fig. 3 show a number of comparisons between the DSI fraction and incubation media from a number of experiments in which oocytes were injected with chick oviduct poly A-rich RNA. The locations on the gels of the major message products ovalbumin (ov) and ovimucoid (om) were identified by immunoprecipitation (Wickens, Woo, O'Malley & Gurdon, 1980).

From Fig. 3 it can be seen that the protein population of the incubation media coincides almost entirely with that of the pellet fraction of the oocyte (endoplasmic reticulum and vesicles) rather than the cytoplasmic (soluble, non-yolk proteins) fraction. Thus, for example, the secreted chick oviduct proteins shown in panel 2A can be found in the DSI fraction (panel 2B). Similarly, the minor polypeptides of the incubation media of panels 2A, 3A and 4A are also found in the oocyte pellet fractions 2B, 3B and 4B. The most prominent of these common, minor species have been arrowed in each panel. It is, of course, also clear that only some of the vesicle fraction proteins are scattered, although this result is to be expected if the fraction contains intracellular structural and


1A
m1
m2
2A
ov
3A
4A
1B
m1
m2
2B
ov
om
3B
4B

enzymatic components associated with the secretory process. The population overlap strongly suggests that the minor polypeptides originate in the oocyte and are secreted (along with any heterologous proteins) into the incubation medium.

Two-dimensional analysis also confirms the conclusion that the minor polypeptides cannot originate from either the follicular cells or from leakage from damaged oocytes. Thus, prior removal of follicle cells does not alter the polypeptide population of the vesicle fraction (compare panels 3B and 4B). Further, the incubation medium from collagenase-treated uninjected oocytes shown in Panel 4A of Fig. 3 contains many of the same minor species found in the incubation media of all other experiments. (Unfortunately, this panel shows a poor fluorograph exposure in which some of the less prominent polypeptides have been lost. However, even with this limitation, the result is consistent with our interpretation.)

Finally, as a precaution, we have also examined the possibility that bacteria or fungi contribute significantly to the population of proteins present in the medium. Thus, in our early experiments aliquots of medium were screened for contamination. Moreover, oocytes were removed from the medium, washed thoroughly and then chased (Berridge & Lane, 1976) with unlabelled methionine. In the absence of further incorporation of radioactive amino acids, the same spectrum of proteins, as judged by one-dimensional gel electrophoresis, was exported for at least another 24 h.

The subcellular location of secreted proteins

To what extent does the protein population of the DSI fraction differ from that of the oocyte cytosol fraction? Previous one-dimensional electrophoretic studies have only been able to discriminate between the two by virtue of the exclusive location of heterologous translation products in the vesicle-enriched fraction. With two-dimensional electrophoresis, we have found that the cytosol

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Fig. 3. Fluorographs from two-dimensional gels of [^{35}S]methionine-labelled oocyte fractions. The pH range of the isolelectric focusing dimension was approximately 4·5–6·8. The molecular weight range is approximately 1500–150000 daltons. *Panel 1.* Oocytes microinjected with guinea-pig mammary gland poly A$^+$ RNA. (A) incubation medium from manually-isolated oocytes. (B) incubation medium from collagenase-treated oocytes. (The major message products caseins A and B are labelled m1 and m2 respectively.) *Panel 2.* Manually-isolated oocytes microinjected with chick oviduct polyA$^+$ RNA. (A) incubation medium. (B) vesicle fraction. *Panel 3.* Manually-isolated control oocytes. (A) incubation medium. (B) vesicle fraction. *Panel 4.* Collagenase-treated control oocytes. (A) incubation medium. (B) vesicle fraction. Most proteins of the incubation medium can be found in their corresponding vesicle fraction and a number of these are indicated (arrows). Incubation medium proteins originating from follicle cells (see text) are labelled (f) and one of these is circled (see legend to Fig. 4). Chick oviduct proteins ovalbumin (ov) and ovomucoid (om) are indicated. Ovomucoid is absent from Panel 2A since extensive glycosylation causes it to migrate with the gel front.

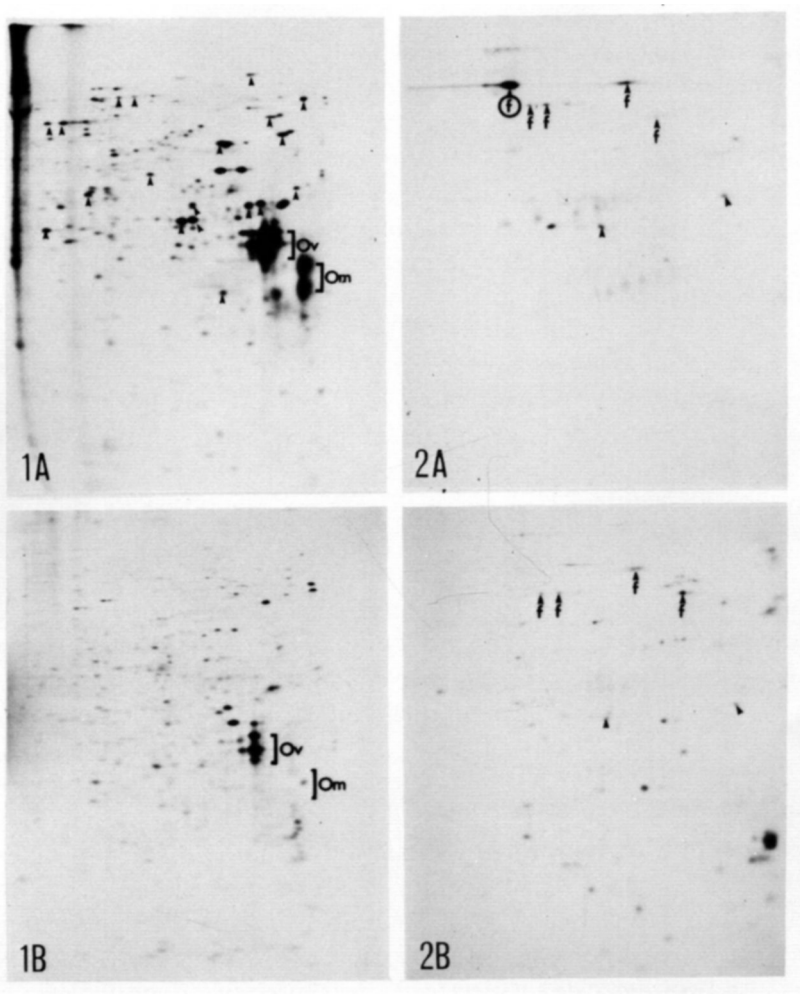
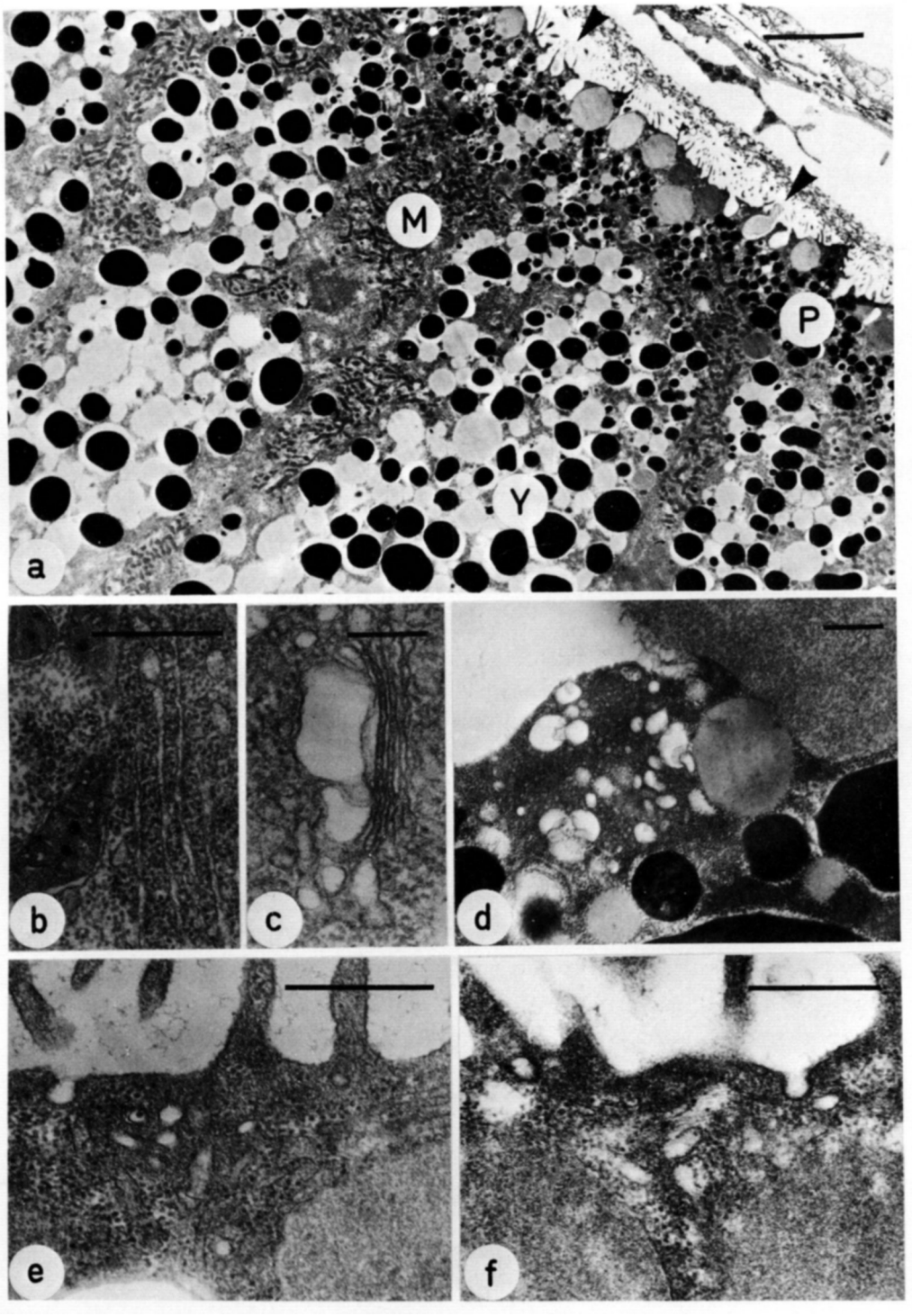


Fig. 4. Fluorographs from two-dimensional gels of oocyte fractions (molecular weight and pH ranges as described in Fig. 3). *Panel 1.* Manually isolated oocytes microinjected with chick oviduct polyA$^+$ RNA. (A) vesicle fraction. (B) cytosol (supernatant) fraction. Chick oviduct proteins ovalbumin (ov) and ovomucoid (om) are indicated. Proteins unique to the vesicle fraction are also shown (arrows). It is apparent that ovomucoid is not miscompartmentalised to the same degree as ovalbumin and is virtually absent from the cytosol fraction. *Panel 2.* (A) incubation medium from manually isolated control oocytes. (B) incubation medium from manually isolated follicle cells (see Results for details). Proteins common to oocyte and follicle cell incubation media are indicated (arrows). Proteins originating from follicle cells are labelled (f). One of these (circled) shows a wide variation in intensity between batches of manually-isolated oocytes. It is absent from the incubation media of collagenase-treated oocytes and isolated follicle cells and could therefore be a polypeptide whose secretion requires the co-operation of both follicle cells and oocytes.

and vesicle fractions show overlapping, but differing polypeptide populations irrespective of messenger injection. Panels 1 A and 1 B of Fig. 4 show a comparison of these two fractions from single oocytes microinjected with chick oviduct message. Most of the polypeptides found in the cytoplasmic fraction (which comprises about 80 % of the radioactive protein within the oocyte) are also found in the vesicle fraction. However, many of the major species in the vesicle fraction are unique to it (Panel 1 A, arrowed). The unique proteins include endogenous secreted species and heterologous translation products made under the direction of injected messenger RNA. The introduction of large amounts of exogenous messenger can lead to the accumulation of the heterologous primary translation products in the cytoplasmic fraction although such 'miscompartmentalized' protein is usually unstable (Lane *et al.*, 1979) and is not available for secretion (Colman *et al.* in preparation). Panel 1 B of Fig. 4 shows an example of this, with different modified forms of ovalbumin being found in both vesicle and cytoplasmic fractions.

Protein secretion by follicle cells

Finally, we have sought to determine whether follicle and theca cells contribute to endogenous secretion by oocytes *in vitro*. We have already shown that collagenase treatment results in the loss of several high molecular weight proteins from the oocyte incubation medium (compare Panels 3A and 4A, Fig. 3 and tracks 5 and 6 of Fig. 2). However, this could be explained in several ways. Either, the presence and cooperation of follicle cells is necessary for the successful secretion of some endogenous oocyte proteins, or the cells surrounding the oocyte also secrete proteins. In an attempt to distinguish between these two explanations, we have compared the incubation media from uninjected oocytes, with and without follicle cells, and we have also incubated isolated follicle and theca cells. A mixture of the latter two cell types were obtained by collagenase treatment of isolated oocytes; the possibility of protein leakage from damaged follicle cells cannot be excluded. Figure 4 shows the incubation media from oocytes with follicular cells (Panel 2A) and that from a mixture of isolated follicle and theca cells (Panel 2B). From previous experiments we have been able to identify those polypeptides lost from the incubation medium through prior collagenase treatment of the oocytes; they are indicated in Panel 2A (labelled 'f'). These same proteins were found in the incubation medium from isolated follicle cells, as were a number of other proteins, including several that were common to both incubation media (arrowed). We therefore, conclude that a number of proteins of high apparent molceular weight that are found in the incubation medium of cultured oocytes result from the presence of layers of cells enveloping the oocytes and originate from these surrounding cells. The possibility exists that their presence arises through active secretion.


M
P
Y
a
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Electron microscopy of oocytes

If the normal oocyte is a secretory cell, we would expect it to contain the cellular machinery for protein synthesis, sequestration and export. We have investigated this possibility using electron microscopy and in particular we have sought to determine whether any morphologically distinct class of secretory vesicles is found in messenger-injected oocytes. Figure 5A shows a low-power view of a section at the animal pole of a control, saline-injected oocyte. This shows that the full-grown oocyte has a complex cytoarchitecture. Immediately beneath the surface membrane is a layer of cortical granules. Beneath this is a layer of small, dark, pigment granules, beneath which the cytoplasm is divided into areas rich in yolk granules, separated by areas rich in mitochondria and other cytoplasmic components. These include granular endoplasmic reticulum (Fig. 5*b*) and many discrete areas of Golgi apparatus (Fig. 5*c*). Examination of the surface of the oocyte (Fig. 5*a*, arrows) shows without doubt that material in the 'cortical granules' is released by the oocyte during *in vitro* culture.

It is important to document the fact that exocytotic material is visible being released by surface vesicles for two reasons. Firstly, there have been several electron microscopic studies of amphibian oocytes which have convincingly demonstrated the presence of endocytotic vesicles involved in the uptake of yolk precursors (Brummett & Dumont, 1977; Wallace, Ho, Salter & Jared, 1973). Secondly, some invaginations into the surface membrane have actually turned out on closer inspection to be a cortical labyrinth of 'subsurface tunnels' (Massover, 1973) which may be immaterial to release or uptake at the oocyte surface.

Several types of vesicle are seen at the surface of messenger-injected oocytes (Figs 5*d*, *e*). Figure 5*d* shows exocytosis of cortical granule contents as well as a condensation of very small, clear vesicles. Similar small vesicles are found in the cortex of control oocytes (5*e*). Coated vesicles, previously shown to be important in endocytosis (Goldstein, Anderson & Brown, 1979) are seen in both control and messenger-injected oocytes (e.g. Fig. 5*f*).

Fig. 5. (*a*) Low-power electron micrograph of animal-pole oocyte cortex. Two vesicles are releasing secretion into the space beneath the vitelline membrane (arrows). P = pigment; Y = areas of yolk (black) and lipid (grey); M = areas rich in mitochondria. Golgi bodies, endoplasmic reticulum and annullate lamellae. (Bar = 5 μm).

(b–f) Higher power electronmicrographs to show granular endoplasmic reticulum (b); Golgi apparatus (c); cortical vesicle in the process of exocytosis and a collection of smaller vesicles, some of which open onto the surface (d); a cortical labyrinth of smooth-surfaced vesicles and tubules (e); coated vesicles (f). (Bar = 0·5 μm) except c, where bar = 0·25 μm.

DISCUSSION

The ability of the *Xenopus* oocyte to synthesize and export heterologous proteins in response to microinjection with exogenous messenger RNA has established it as useful surrogate system in which the mechanisms and controls of sequestration and secretion can be studied. Oocyte secretion is a highly specific process (Colman & Morser, 1979) involving the recognition of nascent secretory polypeptides (Lane *et al.*, 1979), yet it is virtually nonspecific for both species and cell type (Lane *et al.*, in preparation). Significant miscompartmentalization of translation product can be induced under certain conditions as can secretion in the absence of secondary protein modification (Colman *et al.*, in preparation). In the present study, we have sought to characterize further the oocyte secretory system by investigating the role of the accessory cells that envelop individual oocytes.

Our conclusions are drawn from two-dimensional electrophoretic studies and it is important first to establish the limitations of this approach. For example, in the present study, similar oocyte samples show some variation in pattern between experiments and this may be due to a variety of factors. Two-dimensional separations are highly sensitive to minor alterations in the method of sample preparation; differences originating in this way may also be supplemented by those arising from variations in autoradiograph exposure times. Furthermore, we cannot exclude the possibility that heterologous proteins compete with oocyte proteins for export, causing a decrease in the number of species visualized in the incubation medium surrounding message-injected oocytes. For these reasons our experimental approach has been to identify similarities in two-dimensional maps of oocyte fractions and incubation media, no conclusions being drawn from minor differences in patterns. Thus we have identified a number of polypeptides common to the protein populations of incubation media and vesicle-enriched, pellet fractions for both message-injected and uninjected oocytes, irrespective of the presence of their accessory, follicular cells. We conclude, firstly, that secretion by the oocyte is independent of its associated follicular layers and in particular it is unaffected by the complete removal of the intimately associated follicle cell layer. Secondly, the oocyte secretes a large number of endogenous proteins in culture and that, as with heterologous translation products, these proteins are found in the vesicle fraction of the oocyte, presumably prior to export. The kinetic relationship between vesicles and incubation medium has been verified in pulse-chase experiments in which the accumulation of heterologous and endogenous exported protein parallels (but for many species does not exactly match) the decline in sequestered species within the oocyte (data not shown).

The question therefore arises; do *Xenopus* oocytes show endogenous secretion *in vivo*? Certainly, one example of *in vivo* secretion that has been widely documented is the 'cortical granule reaction'. Cortical granules are present in full

grown occytes in widely divergent species (Kemp & Istock, 1964; Szollosi, 1967) and are thought to contain mucopolysaccharide (Anderson, 1968). There contents are released into the perivitelline space on fertilization. It has also been suggested that proteins exported by oocytes may contribute to the formation of extracellular structures such as the vitelline membrane (Dumont & Brummett, 1978). The present studies document the secretory behaviour of oocytes cultured *in vitro* and it remains for further study to establish whether these cells show similar metabolic activities in the animal. Electron microscopy may provide a useful approach to this problem since at least *in vitro*, the mechanism of secretion appears to involve exocytotic release from vesicles.

A final conclusion of the present study is that the vesicle-enriched, pellet fraction of oocytes contains a unique population of proteins including those known to be secreted. We do not yet know whether proteins synthesized under the direction of exogenous mRNA are sequestered into a special class of vesicle within this fraction or whether they share an identical export pathway to that employed for the endogenous proteins secreted by the oocyte.

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