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The *Xenopus* Oocyte as a Surrogate Secretory System

The Specificity of Protein Export

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Combining messenger RNA from one kind of secretory cell with the cytoplasm of another such cell can reveal the nature and specificity of protein export mechanisms. We show that messenger RNAs from secretory cells of chickens, rats, mice, frogs, guinea-pigs, locusts and barley plants, when injected into *Xenopus* oocytes, direct the synthesis and export of proteins. Chicken ovalbumin, *Xenopus* albumin, mouse thyroid-stimulating hormone, locust vitellin and guinea-pig milk proteins were identified using specific antibodies, whilst chicken lysozyme and ovomucoid, rat albumin, *Xenopus* vitellogenin and rat seminal vesicle basic proteins were identified provisionally from their molecular weights. Certain endogenous proteins are sequestered and secreted although most oocyte proteins are not exported. Similarly the major polyoma viral protein and the simian virus 40 and polyoma tumour antigens are retained within the oocyte. Radioactive proteins exported by oocytes programmed with chicken oviduct or *Xenopus* liver RNA are not re-exported in detectable amounts when injected into fresh oocytes, nor is there secretion of chicken oviduct or guinea-pig mammary gland primary translation products prepared using wheat germ extracts. Thus the export of secretory proteins from oocytes cannot be explained by leakage and may require a cotranslational event. The secretory system of the oocyte is neither cell-type nor species-specific yet is highly selective. We suggest that the oocyte can be used as a general surrogate system for the study of gene expression, from transcription through translation to the final subcellular or extracellular destination of the processed protein.

The introduction of messenger RNA from one kind of cell into the cytoplasm of another reveals the general nature of the translational machinery [1–4]. The injection of secretory protein messengers into *Xenopus* oocytes and other cells [5] suggests that the transfer of newly made polypeptides across the endoplasmic reticulum [6, 7] lacks both cell-type and species specificity. Recently it has been shown [8] that oocytes of *Xenopus laevis* will export certain secretory proteins, such as caseins, whose synthesis is directed by heterologous mRNA. This process is highly selective since a non-secretory protein, rabbit haemoglobin, is not exported. In this paper we extend these observations to a range of proteins, secretory and non-secretory, whose mRNAs were derived from a variety of species. We show that whilst secretion is highly selective, it lacks cell-type or species specificity. The demonstration of specific export and specific intracellular localization of secretory proteins recommends the *Xenopus* oocyte as a general system for studying the secretory pathway [9].

Abbreviations. T-antigen, large tumour antigen; t-antigen, small tumour antigen.

MATERIALS AND METHODS

Oocyte Incubation

Oocytes were injected with messenger RNA [1] and cultured [1] in modified Barth x medium [10] (containing additional antibiotics: penicillin 100 units/ml, streptomycin 100 units/ml, gentamycin 70 μg/ml and mycostatin 20 units/ml). Initial experiments included bioassays which revealed little or no bacterial or fungal contamination. After microinjection of the mRNA, which was dissolved in 5 mM Tris pH 7.5 or distilled water, the oocytes were left overnight to permit messenger recruitment [11] and healing of the micropipette wound. After the addition of [^{35}S]methionine

(up to 600 μCi at 6 mCi/ml for 40 oocytes) the batches of from 10–40 oocytes were incubated for 24–48 h. Chase conditions were established [11] by incubating the oocytes for 4 h in modified Barth x medium, which was then made 10 mM in methionine. At each time point during the chase the incubation medium was changed, and about 10 oocytes were fractionated. Throughout the procedures any batches of cells showing the slightest sign of leakage were discarded. Radioactive proteins were injected [7] without prior dilution, dialysis or lyophilization. The injection of proteins exported by oocytes was achieved by collecting medium after about 2 days of incubation in [^{35}S]methionine and, following the addition of non-radioactive methionine (10 mM), introducing this radioactive protein solution into fresh oocytes preincubated (4–16 h) in 10 mM methionine. Oocytes were defolliculated by treatment with collagenase (2 mg/ml in 0.1 M phosphate buffer pH 7.4) for 90 min at 26 °C. Follicle cell removal was confirmed by microscopy.

Analysis

At the end of the incubation the surrounding medium was removed and frozen, whilst the oocytes were fractionated, using homogenization buffer [7] containing 10% sucrose and 150 mM KCl, into supernatant (cytosol), yolk pellet and vesicle extract, the latter is thought to include the contents of both endoplasmic reticulum and secretory vesicles. Cell fractions were run on gels and specific products were precipitated [7] using antibodies raised against chicken ovalbumin [12], *Xenopus* albumin and vitellogenin [13], guinea-pig casein [14], locust vitellin [15], mouse thyroid-stimulating hormone α subunit [16] and simian virus 40 and polyoma tumour antigens and capsid proteins [17,18]. Immunoprecipitates were analyzed on dodecyl sulphate gels. One-dimensional gels were prepared, run and autoradiographed or fluorographed as described [11]: densitometery was performed using a computer-aided integrating gel scanner. Two-dimensional gels were prepared by a modification [19] of O'Farrell's method [20].

Preparation of RNA

RNA, usually made by phenol extraction, was (where stated) enriched for poly(A)-containing species before injection. The method of preparation and final concentration wer as follows: chicken oviduct poly(A)-rich RNA [21] 3.6 mg/ml, *Xenopus* liver RNA from normal and oestrogen-stilulated males or females [11] 5–10 mg/ml, poly(A)-rich rough microsomal and rapidly sedimenting endoplasmic reticulum RNA of rat liver [22] 1.0–1.5 mg/ml, poly(A)-rich locust fat body RNA (using the method of Rhoads [23]) 0.5–2.0 mg/ml, mouse plasmacytoma membrane-bound [24] polysomal RNA 5–10 mg/ml, poly(A)-rich rat seminal vesicle RNA [25] 1.0 mg/ml, poly(A)-rich guinea-pig mammary gland RNA [14] 0.5–1.0 mg/ml, poly(A)-rich mouse thyrotropic pituitary tumour RNA [16] 1.0–2.0 mg/ml, partially purified viral poly(A)-rich RNA from cells infected with polyoma virus or simian virus 40 [17,26] 1.0 mg/ml. Poly(A)-rich barley plant endosperm RNA was prepared by oligo(dT)-cellulose chromatography [27] of membrane-bound [28] polysomes solubilized by heating in 20 mM Hepes pH 7.6, 0.5 NaCl, 1% dodecyl sulphate, 10 mM EDTA for 5 min at 60 °C.

Cell-Free Translation Systems

Nuclease-treated rabbit reticulocyte lysates, with or without added (2.0 A_{280}/ml) dog pancreas membranes [29], were programmed with mouse thyrotropic tumour RNA. Wheat germ cell-free systems were prepared and incubated with guinea-pig mammary gland RNA [14], chicken oviduct RNA [21], or rat seminal vesicle RNA [25].

Preparation of Marker Proteins

Rat seminal vesicle secretory proteins were prepared from normal and [^{35}S]methionine-labelled rat tissues [29], whilst locust vitellin [15], *Xenopus* albumin and vitellogenin [13], guinea-pig caseins [14], and chicken ovalbumin [12] were prepared from unlabelled tissues. [^{35}S]Hordeins [31] were purified from barley plant ears labelled with $^{35}SO_2$.

RESULTS

Oocyte Secretion is Selective yet Lacks Cell Type or Species Specificity

The microinjection of messenger RNAs prepared from a range of different tissues and organisms can be used to define the specificity of the secretory process in *Xenopus* oocytes. Thus frog cells, injected with total or poly(A)-rich RNA from a variety of tissues, were left overnight to permit healing of the injection wound, and were then incubated with [^{35}S]methionine. One or two days later the surrounding medium was analyzed on a dodecyl sulphate gel (Fig. 1) as were the cytosolic and endoplasmic reticulum vesicle fractions of some batches of oocytes (Fig. 4 and 5). The incubation medium from uninjected controls is shown (Fig. 1, tracks 6 and 15) and is similar to that of mock-injected (pricked) controls taken from the same frog; medium from RNA-injected oocytes contains one or more additional radioactive polypeptides. Fig. 1 and 3 show exported proteins from oocytes programmed with RNA from locust fat body (track 2), *Xenopus* liver (female) (track 3), *Xenopus* liver (male)

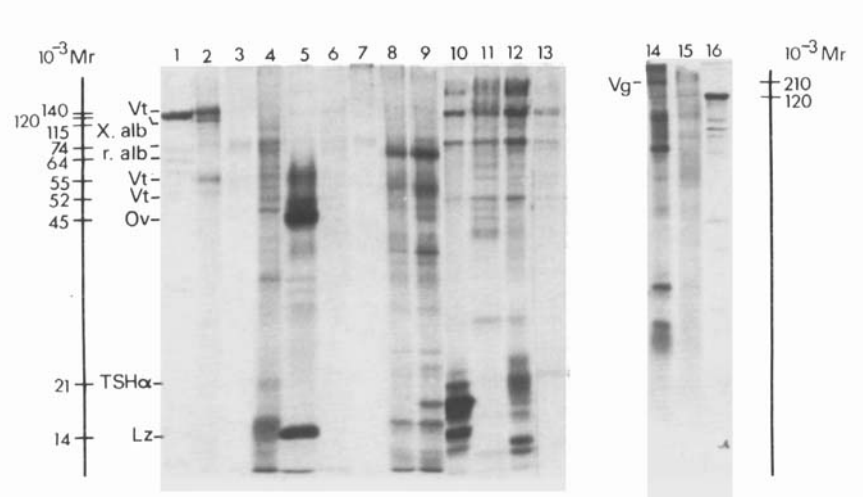

Fig. 1. *Oocyte secretion lacks cell-type or species specificity*. Oocytes were injected with RNA and left overnight: unmarked cells were incubated with [^{35}S]methionine for 24–48 h. Fig. 1 shows electrophoresis on 12½% polyacrylamide gels of the medium surrounding RNA-injected and control oocytes. Tracks 6 and 15 depict medium from uninjected oocytes, track 7 that surrounding defolliculated oocytes and track 11 medium from mock-injected oocytes (taken from a different frog and labelled for longer). Tracks 2–15 include incubation medium from oocytes injected with RNA from tissues of the following types: track 2 locust fat body, track 3 *Xenopus* liver (female), track 4 *Xenopus* liver (male, uninduced), track 5 chicken oviduct, track 8 rat liver rapidly sedimenting endoplasmic reticulum, track 9 rat liver rough microsomes, track 10 rat seminal vesicle, track 12 barley plant endosperm and track 14 *Xenopus* liver (male, estrogen stimulated). Tracks 1 and 16 show adenovirus molecular weight markers (the prominent band being 120000). The molecular weight scale on the left is based on the known molecular weights and mobilities of marker proteins, which, on other gels, have been run alongside the appropriate secreted proteins (which were absent from medium of control oocytes). Markers include frog vitellogenin (Vg), four major locust vitellin species (Vt), chicken ovalbumin (Ov) and lysozyme (Lz), thyroid-stimulating hormone (TSHα, prepared in the membrane-supplemented reticulocyte lysate), rat albumin (r.alb) and *Xenopus* albumin (X. alb , major component). The molecular-weight scale on the right refers to tracks 14–16, which come from a different gel

track 4), chicken oviduct (track 5), rat liver rapidly sedimenting endoplasmic reticulum (track 8) and rough microsomes (track 9), rat seminal vesicle (track 10), barley plant endosperm (track 12), *Xenopus* liver (male, oestrogen-stimulated) (track 14), guinea-pig mammary gland (tracks 1, 2 and 3 of Fig. 3) or mouse plasmacytoma cells (tracks 6, 7 and 8 of Fig. 3). The identity of several of the exported polypeptides has been established by gel analysis of immunoprecipitates obtained with specific antibodies (Fig. 2) indicating that the additional proteins are made under the direction of the heterologous messenger RNA. Species identified by immunoprecipitation include guinea-pig caseins A and B (Fig. 2A) chicken ovalbumin (multiple forms, Fig. 2B), *Xenopus* albumin (Fig. 2C), the α subunit of mouse thyroid-stimulating hormone (Fig. 2D), and insect vitellogenin (Fig. 2E). Chicken lysozyme was recognised by its specific binding to formalin-treated *Staphylococcus aureus* envelopes (Colman and Bamhra, unpublished) whilst ovomucoid was identified using antiovomucoid serum (data not shown). As indicated in Fig. 2, with the exception [7] of caseins A, B and C, all immunoprecipitated heterologous translation products electrophoresise with marker proteins secreted by the appropriate foreign tissue.

Preliminary identification of several other polypeptides, including rat albumin, rat seminal vesicle fast and slow basic proteins, and *Xenopus* vitellogenin, has been achieved by comparing the molecular weights of the major processed secretory species exported by a particular cell type with the major new species exported by oocytes injected with RNA from that tissue (Fig. 1). Vitellin prepared from locust eggs yields four polypeptides which electrophorese with four (immunoprecipitable) polypeptides exported by the oocyte (Fig. 1, track 2 and Fig. 2E). The identification of these four polypeptides by direct immunoprecipitation is considered preliminary, for similar bands, albeit faint, are detectable in the medium from control oocytes, presumably as a result of non-specific adsorption to added carrier vitellin. Vitellin is thought to contain at least four minor polypeptides [32], and indeed four faint bands of about the expected molecular weights, are seen on the gel track of oocyte medium. Oocytes injected with semnal vesicle RNA export two proteins that electrophorese with the fast and slow basic proteins secreted by methione-labelled rat gland cells: the corresponding wheat germ products, identified as such by immuno-precipitation (data not shown), have higher apparent molecular weights (Fig. 2F). The fast component from oocytes, seen most clearly in Fig. 1, track 10, has an apparent molecular weight of about 14000 on our gel system [30]. Mouse plasmacytoma RNA directs the export of major (M_r 55000 approximately) and minor (M_r 25000 approximately) species that have not as yet (see Table 1, however) been identified as immunoglobulin chains (Fig. 3, tracks

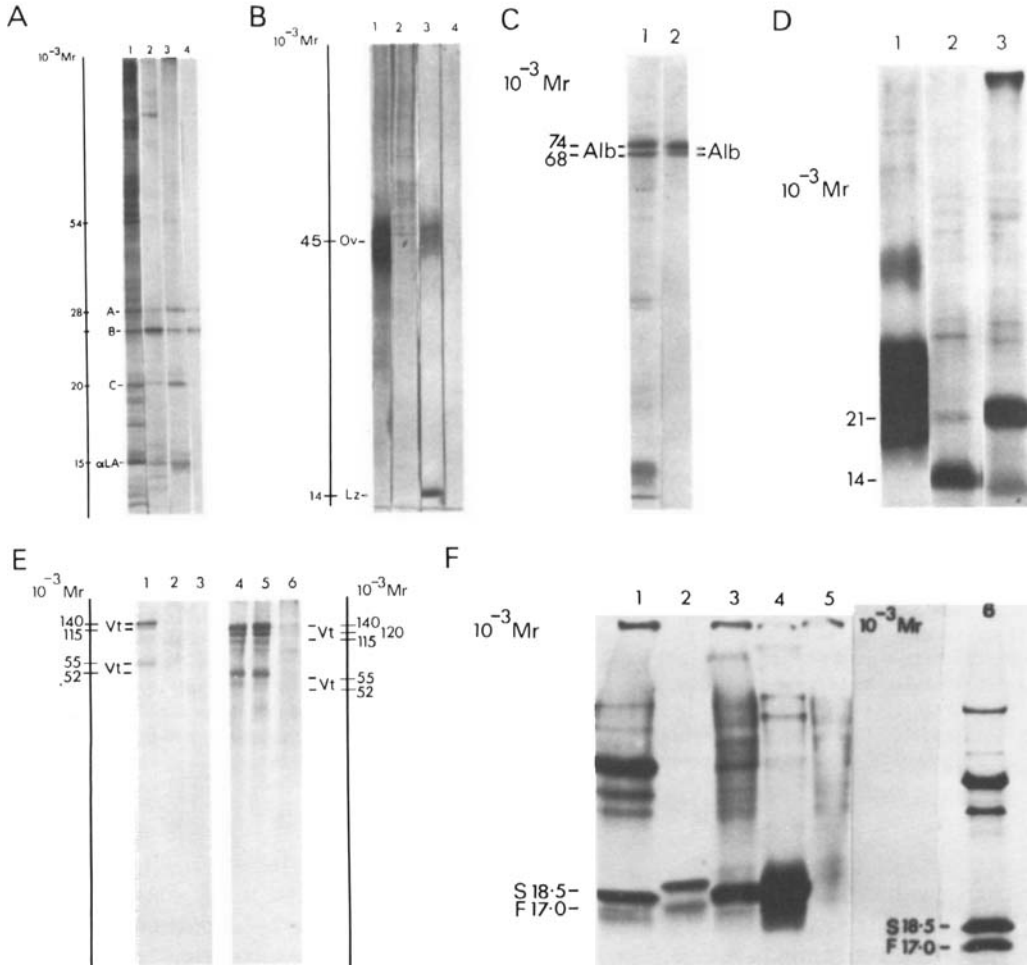

Fig. 2. *The identification of heterologous secretory proteins exported from* Xenopus *oocytes.* Oocytes were injected with messenger RNA and, after incubation with [^{35}S]methionine for 24–48 h, the surrounding medium was immunoprecipitated. The gradient gel ($12^1/_2$–$22^1/_2$ %) shown in (A) analyses the following combinations from oocytes injected with mammary gland RNA: track 1 oocyte homogenate, track 2 incubation medium, track 3 anticasein immunoprecipitates of oocyte homogenate, and track 4 anticasein immunoprecipitation of medium. The gradient gel in (B) shows antiovalbumin immunoprecipitates from the following: track 1 oviduct-RNA-injected oocyte, track 2 mock-injected oocyte, track 3 medium from RNA-injected oocyte and track 4 medium from mock-injected oocyte. (A, B) Indirect immunoprecipitation using *Staphylococcus aureus* protein A [18]. (C) (Linear gel) shows medium from oocytes injected with *Xenopus* liver poly(A)-rich RNA before (track 1) and after (track 2) immunoprecipitation with antialbumin serum. The mobility of marker *Xenopus* albumin (Alb), which runs as a doublet (M_r 74000 and 68000) is denoted by bars. The gel tracks shown are from different gels. (D) (Linear gel) shows precipitates obtained with anti-(ovine luteinizing hormone α subunit): track 1 medium from oocytes injected with mouse pituitary tumor RNA (medium from control oocytes lacked specific immunoprecipitable bands), track 2 marker of reticulocyte lysate programmed with mouse pituitary tumour RNA and track 3 marker of lysate supplemented with dog pancreas membranes plus mouse pituitary tumour RNA. Molecular weights are known for the processed α subunit (M_r 21000) and its precursor (M_r 14000). (E) Track 1 shows medium surrounding oocytes injected with locust fat body RNA: track 2 depicts medium from control-injected (oocytes plus degraded RNA of frog liver) cells, whilst track 3 show that surrounding uninjected oocytes. Tracks 4–6 show immunoprecipitates obtained with added carrier vitellin, anti locust egg vitellin antibodies and oocyte incubation medium (tracks 4 and 5, two batches of locust RNA injected oocytes and track 6 uninjected oocytes). (F) (Linear gel) shows a comparison of rat seminal vesicle proteins exported from RNA-injected oocytes (track 4) with proteins exported from both labelled (track 1) and unlabelled (track 6) seminal vesicle cells. The medium from uninjected oocytes lacks several polypeptides including those electrophoresing with the fast (F) and slow (S) basic proteins: the latter are also detectable within RNA injected (track 3) but not control (track 5) oocytes. The RNA used for injection was also translated in the wheat germ system and the two major products formed (track 2) were shown to be fast and slow seminal vesicle proteins by means of specific antibodies (see Results). The fast and slow basic proteins have been assumed [30] to have molecular weights of 17500 and 18500 respectively (see Results)

6–8). Only the protein of molecular weight 25000, which seems to be exported rather slowly, can be seen within the vesicle fraction (Fig. 4, tracks 6 and 7). Oocytes programmed with oviduct RNA do not secrete (Fig. 1 track 5) any major protein of the mobility expected for chicken conalbumin (M_r 76000) but do export significant amounts of a protein (M_r 58000) of unknown glycosylation state and identity. Barley proteins exported (Fig. 1, track 12) in response to injected endosperm RNA do not display the gel

mobility or propan-2-ol solubility characteristics of hordeins, although the RNA used codes for such storage proteins both *in vivo*, where hordeins are found transiently within the oocyte vesicle fraction, and *in vitro* (data not shown).

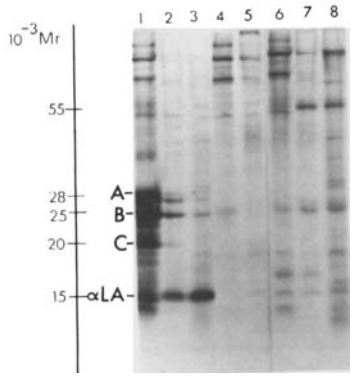

Fig. 3. *The export of guinea-pig milk and mouse plasmacytoma proteins made in oocytes under the direction of injected RNA.* Oocytes were injected with RNA, left overnight and then selected cells were incubated with [^{35}S]methionine for 24 h. After removal of the medium some of the oocytes were fractionated (Materials and Methods) whilst others were incubated in the presence of 10 mM methionine. After 24 h the medium was changed, more oocytes were fractionated and the incubation continued, further samples being taken at 48, 72 and 96 h. Gel analysis of incubation media from some of the earlier samples is shown: tracks 1–3 (mammary gland) and 6–8 (mouse plasmacytoma) RNA-injected and tracks 4 and 5, uninjected oocytes; tracks 1, 4 and 6, [^{35}S]methionine labelling (24 h), tracks 2, 5 and 7, 10 mM methionine (24 h) and tracks 3 and 8 (48 h). Vesicle fractions from this pulse-chase experiment are shown in Fig. 4

The Fate of Secretory and Non-secretory Proteins Made in Oocytes under the Direction of Injected Messenger

Heterologous secretory proteins found within the oocyte are mainly present in the vesicle fraction. Thus Fig. 4 depicts the sequestration of species identified provisionally as frog vitellogenin and lipovitellin, chicken ovalbumin, guinea-pig milk proteins, mouse plasmacytoma proteins and locust vitellogenin. All except the mouse and locust proteins have been identified by immunoprecipitation, as shown (tracks 13 and 14) for frog vitellogenin and lipovitellin. If oocytes containing radioactive milk proteins are chased with unlabelled methionine the milk proteins disappear from the vesicle fraction. Fig. 4 shows that the caseins disappear faster than α-lactalbumin. The caseins also accumulate more rapidly in the incubation medium (Fig. 3). A similar experiment (Fig. 3 and 4) shows that different mouse plasmacytoma proteins also seem to be exported at different rates.

The injection of a messenger coding for a non-secretory protein, such as globin, causes synthesis and retention [8] of the hetrologous protein within the cytosol [6]. We have extended this observation to a number of non-secretory simian virus 40 and polyoma proteins. Thus structural proteins and tumour antigens could not be detected outside the oocyte: Table 1 shows that more than 50 and often more than 100 times as much of each viral protein was present inside as opposed to outside the oocyte. Similar results were obtained with viral proteins, including large and small tumour antigens, made under the direction of simian virus 40 DNA introduced into the oocyte nucleus (Table 1).

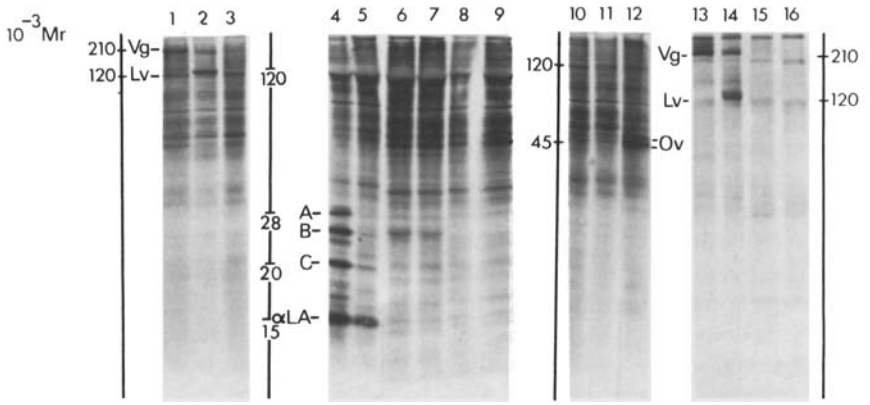

Fig. 4. *The sequestration within a vesicle fraction of proteins made in oocytes from heterologous and homologous messenger RNAs.* Batches of defolliculated and normal oocytes, some injected with RNA, were incubated in [^{35}S]methionine for 24 h before fractionation into yolk, cytosol and vesicle contents. In most experiments, cells were also fractionated after chasing for 24 h with 10 mM methionine. Vesicle contents were analysed on 12½% polyacrylamide gels: thus track 1 shows vesicles from oocytes injected with locust fat body RNA, track 2 estrogen-stimulated male *Xenopus* liver RNA, track 3 uninjected control oocytes, tracks 4 and 5 guinea-pig mammary gland RNA, track 5 24-h chase, tracks 6 and 7 mouse plasmacytoma RNA track 7 24-h chase, tracks 8 and 9 uninjected control, track 9 24-h chase, track 10 whole oocyte, track 11 defolliculated oocyte and track 12 chicken oviduct RNA. Tracks 13–16 show anti-vitellogenin immunoprecipitates of vesicle contents: track 13 estrogen-stimulated male *Xenopus* liver RNA (4-h incubation), track 14 24-h chase and tracks 15 and 16 uninjected controls, similarly pulsed and chased. Fig. 4 is a composite of four different gels, and the apparent molecular weights and mobilities of marker proteins, given alongside each gel are as follows: frog vitellogenin (Vg) 210000; frog lipovitellin (Lv) 120000 and chicken ovalbumin (Ov) 45000. The oocyte-synthesized milk proteins (denoted A, B, C and αLA) have been identified [7] as modified caseins A, B, C and α-lactalbumin

Table 1. *The retention of viral proteins within oocytes injected with RNA from cells infected with polyoma or simian virus 40 (SV40) or with SV40 DNA*

Injected and uninjected cells were labelled with [^{35}S]methionine for 24–48 h before the immunoprecipitation (iptn) of viral proteins present in oocytes and their surrounding medium. SV40 DNA injected Oocytes [75] were incubated for 24 h before labelling. Immunoprecipitates were analyzed on dodecyl sulphate gels and the amounts of viral protein 1 (VP1), T-antigen and t-antigen were measured by densitometery. Affinity-purified (Paucha, unpublished) simian virus 40 t-antigen messenger was also injected into oocytes and the distribution of its products measured as described above. In all the experiments shown in Table 1 no viral proteins could be detected outside the oocyte, as judged by gel autoradiography: thus calculations of the ratio of products inside and outside are based on the smallest amount that could be detected by densitometry

Source of injected RNA	Oocyte-synthesized protein	Criterion of identity	Amount inside oocyte / Amount outside oocyte
Polyoma-infected cells	VP1	elec. with VP1; iptn	> 50
Polyoma-infected cells	T-antigen	elec. with T-antigen iptn	> 50
Affinity-purified t-antigen mRNA from SV40-infected cells	t-antigen	elec. with t-antigen iptn	> 100
SV40-infected cells	T-antigen	elec. with T-antigen; iptn	> 30
SV40 DNA (nuclear injection)	VP1	elec. with VP1	> 50
	T-antigen	elec. with T-antigen; iptn	> 100
	t-antigen	elec. with t-antigen; iptn	> 100

The Subcellular Localization and Secretion of Endogenous Oocyte Proteins

Selectivity can be exerted at several stages along the secretory pathway: study of the oocytes own proteins by subcellular fractionation combined with two-dimensional gel electrophoresis reveals some of the different levels at which selection can occur. The folliculated oocyte synthesizes a wide variety of proteins, and nearly all the species resolved on gels are also made by defolliculated oocytes (Fig. 5D and I): the contribution of the surrounding follicle or theca cells [33] appears slight. The cytosol, which contains about 80% of the newly made protein, yields a pattern (Fig. 5E) similar but not identical to that of the whole oocyte. The vesicle fraction (Fig. 5F) contains a population of proteins quite different from those of the cytosol. Heterologous secretory proteins are also distributed unequally between the two fractions, as shown for guinea-pig caseins A and B in Fig. 5G and J. The caseins can barely be detected in the cytosol. Proteins made within the oocyte can account both qualitatively (Fig. 5F and H) and quantitatively for the vesicle contents. Selectivity also seems to be exerted at the level of secretion, as shown by the restricted number of proteins present in the incubation medium from mammary-gland-RNA-injected (Fig. 5B) or uninjected (Fig. 5A) oocytes. Milk proteins were identified provisionally from the molecular weights [7] of the immunoprecipitable oocyte products: two-dimensional gel electrophoresis of vesicles and medium reveals several charge variants, which are probably of natural origin given the absence of such variants amongst other proteins. At least some of the endogenous secreted proteins come from the oocyte itself, as shown by analysis (Fig. 5C) of medium surrounding defolliculated oocytes. Isolated follicle and theca cells also export proteins.

Proteins Injected into Oocytes are not Exported Rapidly

The signal hypothesis [34] predicts cotranslational transfer of the newly synthesized polypeptide across the endoplasmic reticulum: later processes, such as secretion, will thus appear dependent on translation. The injection of radioactive proteins into oocytes permits the study of such requirements of the secretory process. It is often difficult to make concentrated solutions of native radioactive proteins: fortunately the oocyte itself can be used to prepare labelled proteins suitable for injection. Fig. 6 shows that, as judged by autoradiography, proteins exported by oocytes injected with liver RNA are not re-exported when introduced into fresh oocytes. Proteins secreted by oocytes and their surrounding [33] follicle and theca cells are also not re-exported following microinjection. Similarly, the many different proteins, ovalbumin included, from the medium surrounding oocytes injected with chicken oviduct RNA, are retained following reinjection. Even primary translation products made in the wheat germ system under the direction of mammary gland or oviduct RNA do not leave the oocyte in detectable amounts (over a 20-h period): whilst the milk protein primary products are degrade rapidly ($t_{1/2}$ = 0.6 h), ovalbumin ($t_{1/2}$ = 22 h) is quite stable [7]. Most processed secretory proteins are stable in oocyte cytoplasm: the value calculated from Fig. 6 for *Xenopus* albumin ($t_{1/2}$ = 12 h) is typical, although interferon [8] and the low-molecular-weight reinjected protein ($t_{1/2}$ = 1.5 h), seen in Fig. 6

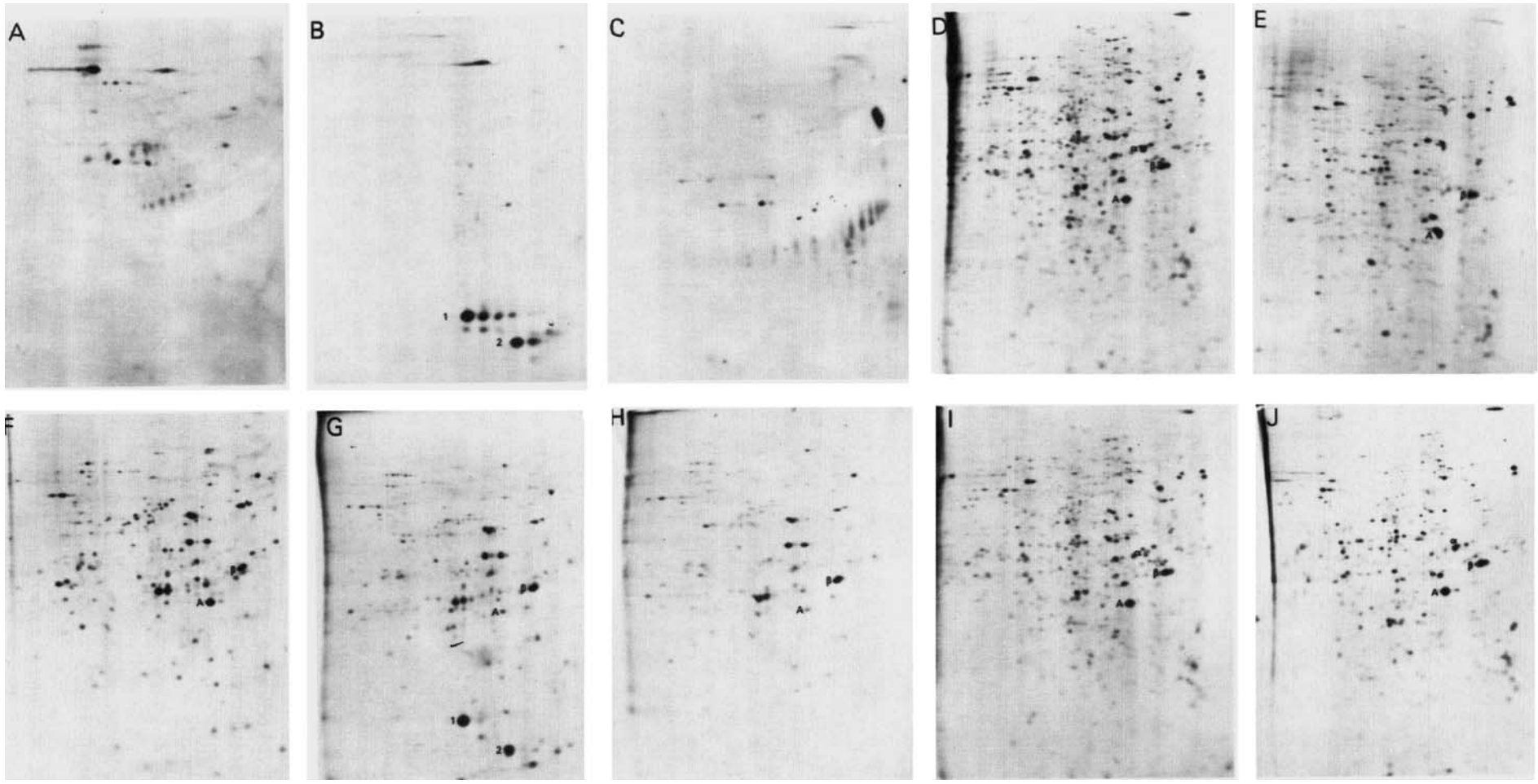

Fig. 5. *The selectivity of sequestration and secretion of endogenous* Xenopus *oocyte proteins.* Batches of normal and defolliculated oocytes, some injected with mammary gland mRNA, were incubated in [^{35}S]methionine for 20 h, fractionated and then analysed by 2-dimensionalgel electrophoresis. Thus (A) shows incubation medium (folliculated oocytes), (B) medium (RNA-injected folliculated oocytes), (C) medium (defolliculated oocytes), (D) defolliculated whole oocytes, (E) cytosol (folliculated oocytes), (F) vesicles (folliculated oocytes) (G) vesicle (RNA-injected defolliculated oocytes), (H) vesicles (defolliculated oocytes), (I) folliculated whole oocytes and (J) cytosol (RNA-injected folliculated oocytes). The reproducibility of the patterns obtained was established by repeating the experiment using oocytes from a different frog: only minor variations were seen (in this experiment oviduct RNA was used for the injected samples). All gels were 10% polyacrylamide. Actin (A) and tubulin (α and β) were identified by electrophoresis with marker proteins [19, 76], whilst the symbols 1 and 2 indicate proteins of the apparent molecular weights (M_r 27000 and 24000) of immunoprecipitable oocyte caseins A and B

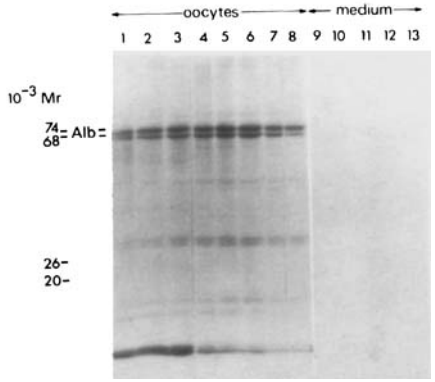

Fig. 6. *The retention of secretory proteins injected into* Xenopus *oocytes*. Oocytes were injected with poly(A)-rich liver RNA (from male frogs), were left overnight, and then incubated with [^{35}S]-methionine for 48 h. Equal amounts of the surrounding medium was then injected into batches of fresh oocytes: at various times batches were analysed for the presence of radioactive proteins in both incubation medium and oocytes. Numbers of oocytes and incubation times for tracks 1–8 (oocyte homogenate) and 9–13 (incubation medium) are as follows: (1 = 0 min, co-homogenization with about twice the volume injected (1.5 oocytes), 2 = 1 min, (2.6 oocytes), 3 = 2 min (2.8 oocytes), 4 = 40 min (2.2 oocytes), 5 = 90 min (1.8 oocytes), 6 = 265 min (2.4 oocytes), 7 = 645 min (3 oocytes), 8 = 1100 min (3 oocytes), 9 = 40 min (3 oocytes), 10 = 90 min (3 oocytes), 11 = 265 min (3 oocytes), 12 = 645 min (3 oocytes) and 13 = 1100 min (3 oocytes). Thus tracks 1–8 were run at constant radioactivity per gel slot, as were tracks 9–13. After normalization and correcting for the different amount applied per slot the half-life of degradation of the injected proteins can be calculated by densitometery [7]. Frog liver albumin (Alb) was identified as a prominent doublet (M_r 74000 and 68000), absent from medium surrounding control oocytes, precipitated by antibodies raised against purified *Xenopus* albumin (Fig. 2C)

near the bottom of the gel, are unstable. Thus the microinjection of secretory proteins reveals the selectivity of the export process and, in accordance with the signal hypothesis [34], suggests a need for some contranslational event.

DISCUSSION

The export of proteins made in frog cells under the direction of injected messengers from chickens, guinea-pigs, locusts, rats, mice and plants suggests the secretory mechanism lacks species specificity. Moreover the secretion by the oocyte of proteins characteristic of nine differentiated tissues (Tables 2) implies [8] that the mechanism also lacks cell-type specificity. The possibility that cell-type-specific restrictions exist but are in themselves species-specific is ruled out by the export from *Xenopus* oocytes of *Xenopus* liver proteins. Bacteria [35] as well as oocytes can export chicken ovalbumin and, when considered with other results obtained using the frog cell system, this suggests that a least some secretory mechanisms are common to all cells.

The general nature of the machinery governing both the sequestration and export of proteins is combined with great selectivity. Thus globin made from injected messenger is found in the cytosol [6] and can hardly be detected [8] in the surrounding medium, even when the same oocytes are exporting caseins. Moreover, heterotopically synthesized [7] cytosolic ovalbumin is not exported from ovalbumin-secreting oocytes. The distribution of the products of polyoma viral protein 1 mRNA is similar, the amount outside the oocyte being less than 2% of that within. Analogous results (Table 1) have been obtained with simian virus 40 and polyoma tumor antigen messengers: immunoprecipitation revealed T and t-antigens within the oocyte. Rat liver proteins made under the direction of injected rapidly sedimenting endoplasmic reticulum RNA also reveal selective export, for although albumin and at least fifteen other proteins are found outside the oocyte (Fig. 1), immunoprecipitation shows that cytochrome P-450 and epoxide hydratase are retained, inserted into membranes within the frog cell. Rat albumin is sequestered, but not inserted, within vesicles and is subsequently secreted (Ohlsson, Lane and Guengueritch, unpublished). Thus entry into the vesicle fraction appars a necessary but not sufficient condition for protein export, whilst all our results suggest that a signal sequence [34] is required for vesicularization [7] and secretion.

The results of two-dimensional gel analysis of vesicles, cytosol and incubation medium from RNA-injected, normal and defolliculated oocytes are in accordance with these principles: thus the vesicle pattern is more complex than that of the incubation medium, and very different from that of the cytosol. Moreover, heterologous secretory proteins, such as caseins A and B [14], are sequestered (and exported) but are barely detectable in the cytosol. It should also be noted that there is extensive overlap between the endogenous proteins of defolliculated oocytes present in vesicles and incubation medium, suggesting a kinetic relationship between some of the proteins in these two fractions.

The microinjection of radioactive secretory proteins reveals selectivity of a different kind. Our earlier work [7] has shown that injected secretory proteins, with or without their signal sequences, cannot be detected within endoplasmic reticulum vesicles. The failure of injected proteins to be exported does not stem from rapid degradation: nor does it result from the absence of a signal sequence or the presence of carbohydrate residues. Although proteins bearing detachable signal sequences are rapidly degraded [7], the stable ovalbumin primary translation product, which contains an internal signal sequence [36] and lacks carbohydrate, is not exported. Thus all our results are compatible with the signal hypothesis [34] which predicts that only nascent proteins can be transferred across the endoplasmic reticulum and thus enter the secretory pathway. The retention within the

Table 2. *The specificity of protein export from* Xenopus *oocytes*
Secreted proteins (designated +) are defined as those detectable in the medium surrounding RNA injected but not control (uninjected or mock injected) oocytes. Proteins were identified by the following criteria: molecular weight, electrophoresis (elec.) with marker proteins and immunoprecipitation (iptn)

Source of RNA	Protein	Criterion of identity	Export (+) or retention (−)
Chicken oviduct	ovalbumin	iptn and elec. with ovalbumin	+
	conalbumin	M_r	+
	lysozyme	elec. with lysozyme	+
	ovomucoid	M_r	+
Locust fat body	4 vitellin polypeptides	iptn and elec. with 4 vitellin polypeptides	+
Guinea-pig mammary gland	casein A	iptn	+
	casein B	iptn	+
	casein C	iptn	+
	α-lactalbumin	elec. with α-lactalbumin	+
Mouse thyrotropic pituitry tumours	thyroid stimulating hormone (α-subunit)	iptn, elec. with α subunit	+
Mouse plasmacytoma cells	M_r 25000 and 55000	−	+
Honey-bee venom gland	Promelittin[a]	elec. with promelittin, peptide analysis	slow or −
Defolliculated oocyte (uninjected)	endogenous oocyte proteins	2-D gel analysis	+
Folliculated oocyte (uninjected)	endogenous oocyte and follicle cell proteins	2-D gel analysis	+
Rat seminal vesicle	basic proteins	elec. with basic proteins	+
Rat liver rapidly sedimenting endoplasmic reticulum	albumin	M_r	+
Rat liver rough microsomes	albumin	M_r	+
	epoxide hydratase[d]	iptn and elec. with epoxide hydratase	−
	cytochrome P-450[d]	iptn and elec. with cytochrome P-450	−
Barley plant endosperm	unknown		+
Xenopus liver	albumin	iptn and elec. with albumin	+
Xenopus liver (oestrogen-stimulated)	vitellogenin albumin	elec. with vitellogenin	+
Human lymphoblastoid cells	interferon[b]	bioassay	+
Mouse myeloma cells	immunoglobulin light chain[c]	iptn and elec. with light chain	+
SV40-infected cells	T and t-antigens	iptn and elec. with T and t-antigens	−
Polyoma-infected cells	VP1	iptn and elec. with VP1	−
	T and t-antigens	iptn and elec. with T and t-antigens	
Rabbit reticulocyte	globin[b]	M_r	−
Reovirus-infected cells	viral proteins[e]	M_r	−

[a] Lane, Champion, Haiml and Kreil unpublished.
[b] [8].
[c] Colman and Williamson unpublished.
[d] Ohlsson, Lane and Guengueritch, unpublished.
[e] Colman and Morser unpublished.

oocyte of stable injected proteins is a powerful argument against leakage as an explanation of protein export. Leakage of protein from mRNA injected oocytes is rendered still more unlikely by the experimental design, which includes a wound-healing period followed by stringent selection of dishes containing only intact oocytes.

The oocyte and egg translate a wide range of secretory protein mRNAs [4,6−8,11,16,37−73] and carry out a variety of post-translational modifications [4]. With few clear exceptions, namely the cleavage of mouse β-glucoronidase [74], the iodination of thyroglobulin [58] and the conversion of promelittin to melittin [37] and of proinsulin to insulin [50], the oocyte has so far proven capable of modifying the proteins of specialized cells in their characteristic manner. Indeed this is one advantage of the oocyte as a surrogate secretory system. Doubtless other ex-

ceptions will be found but, apart from the anomolous mobility [7] of both sequestered and secreted (Fig. 2–4) oocyte milk proteins, none are obvious within the range of proteins studied. The conversion of locust vitellogenin to four major polypeptides that electrophorese with major authentic vitellin species contrasts with the failure of the oocyte to cleave promelittin, another insect protein. The locust vitellogenin messenger codes intitially for a high-molecular-weight species [32] and indeed a protein (usually a doublet) of approximately 250000–220000 M_r is found in the oocyte vesicle fraction (Fig. 4, track 1). Frog vitellogenin messenger also codes for such a sequestered species which, unlike locust vitellogenin, is perhaps secreted (Fig. 1, track 14) as a relatively stable protein of about 210000 M_r. Processed lipovitellin and phosvitin are finally recovered [11] within the yolk platelets of RNA-injected oocytes, and we now believe this stems from the uptake of exported *Xenopus* vitellogenin. As shown in Fig. 4, some lipovitellin, made from injected frog messenger, is recovered in the vesicle extract: however, vesicles are contaminated with yolk and since intact vitellogenin is found in yolk platelets the latter are the likely site of cleavage.

The secretion of proteins by oocytes in culture is of unknown biological significance. It has been proposed [6] that sequestered proteins are stored within the oocyte for use during embryogenesis. The export of endogenous oocyte proteins continues for days or weeks in culture: thus a storage hypothesis remains tenable despite [8] the rapid secretion of many foreign proteins, for it is clear from Fig. 3 that different proteins are exported from the vesicles at different intrinsic rates.

Despite being of unknown significance the oocyte secretory system provides an opportunity to study both the mechanism and control of sequestration and export. Experiments can be designed to reveal aspects of the injected substance, or of the recipient cell: thus oocytes have often been used for messenger assays or experiments involving a biologically active or highly processed end-product [4, 74], in particular for measurements of interferon mRNA activity [73]. The general nature and efficiency of oocyte secretion may aid such studies and permit the preparation of highly labelled biologically active proteins. Whilst the specificity and regulation of secretion can be investigated using the whole cell, cell-free systems prepared from oocytes should permit vectorial transfer to be analyzed in detail. The lack of species or cell type specificity shown by the secretory apparatus of the oocyte means that in a given cell type one can compare the formation and export of different proteins. Such a surrogate system can resolve the separate influences of messenger and secretory apparatus. The frog cell has already been used to study errors of compartmentation and possible corrective mechanisms [7]. The oocyte may prove to be a general system for the analysis of the factors influencing subcellular localization, not merely of secretory proteins but of components within other structures, and will thereby provide a more complete system for the analysis of gene expression [75].

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