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The Fate of *Xenopus* and Locust Vitellogenins Made in *Xenopus* Oocytes

An Export-Import Processing Model

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Xenopus liver or locust fat body RNA injected into *Xenopus* oocytes directs the formation of a high-molecular-weight species which is found transiently within the oocyte in a membranous vesicle fraction. The conversion of such internally generated *Xenopus* vitellogenin to yolk platelet proteins is reduced by the presence in the incubation medium of anti-vitellogenin antibodies, or of tunicamycin, or of a mixture of colchicine and cytocholasin, but neither export nor conversion is blocked by removal of the follicular layers which surround the oocyte. Moreover, surrounding mRNA injected defolliculated oocytes with an excess of small defolliculated oocytes leads to a reduction in lipovitellin accumulation in the cells making vitellogenin and the appearance of lipovitellin in the platelets of the uninjected feeder cells.

We propose that newly-made locust and *Xenopus* vitellogenins are sequestered in vesicles and are then secreted by the oocyte, the locust species being further modified by cleavage just before export. Thus processing of heterologous yolk precursors follows the pathway characteristic of the cell type used to prepare the donor RNA. We suggest that the import mechanism discriminates between the exported locust and frog proteins, and that only the *Xenopus* vitellogenin subsequently enters the oocyte, where it is converted to lipovitellin and phosvitin by an enzyme present in the yolk platelets. Thus we explain the apparently paradoxical observations that internally generated and externally supplied frog vitellogenin are both converted to yolk platelet proteins, whilst injected vitellogenin is not.

The injection of informational macromolecules into the nucleus or cytoplasm of the *Xenopus* oocyte permits study of the specificity and control of gene expression, from transcription [1], through translation [2], to the final subcellular [3] or extracellular [4] destination of the processed protein. The synthesis of *Xenopus* vitellogenin within *Xenopus* oocytes is of particular interest, for the fate of the internally generated species can be compared to that of vitellogenin entering by the normal pinocytotic pathway [5, 6]. Somewhat surprisingly vitellogenin from both sources is converted to platelet-bound phosvitin and lipovitellin. In contrast, *Xenopus* vitellogenin injected directly into oocytes is not converted to yolk platelet proteins [7] but is slowly degraded [8]. Vitellogenin made in oocytes under the direction of injected messenger RNA is first detected within membranous vesicles [3], as is absorbed vitellogenin [9, 10].

In the present study we have investigated the pathway within the *Xenopus* oocyte which leads proteins originating in different cellular compartments to the same final destination. Moreover the species specificity of several steps along this route has been evaluated by examining the fate of locust vitellogenin generated within the *Xenopus* oocyte subsequent to mRNA injection. The disparate fates of *Xenopus* and locust vitellogenins made in *Xenopus* oocytes is discussed in terms of substrate-specific processing and of an export-import model.

MATERIALS AND METHODS

Injection and Culture of Xenopus *Oocytes*

Oocytes were injected with RNA [2] and were cultured in modified Barth X medium [11] containing antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml, mycostatin 20 U/ml, gentamycin 70 μg/ml) for 24 h to permit healing of the micropipette wound and messenger recruitment [8]. Oocytes were defolliculated by overnight incubation at 19 °C in Barth X containing collagenase (Sigma Biochemicals, Slough, UK) at 4 mg/ml, followed by manual removal of adhering follicular layers, after which the incubation in collagenase was continued for a further 4 h. The oocytes were then labelled with [^{35}S]methionine (5 μCi/oocyte, 5 mCi/ml at 150–300 Ci/mol). RNA injected and control oocytes treated with tunicamycin (20μg/ml), or with colchicine (5 mM) plus cytocholasin (25 μg/ml) were preincubated with these drugs for 16 h before the addition of radioactive methionine. Throughout these procedures dishes of oocytes showing signs of leakage were discarded [4].

Subcellular Fractionation and Analysis of Newly-Made Proteins

After the incubation, the surrounding medium was collected and the oocytes fractionated by a single-step procedure [12]. Thus the frog cells were homogenized in buffer T (20 mM Tris pH 7.6, 50 mM KCl and 10 mM MgAc) containing additional 100 mM KCl, 300 mM sucrose and 0.12 mM phenylmethylsulfonyl fluoride and were layered above a 1-ml cushion of 600 mM sucrose (in buffer T). After centrifugation at 10000 × *g* for 15 min at 4 °C the supernatant (cytosolic fraction) was collected. The pellet was extracted twice with 200 μl of phosphate-buffered saline (120 mM NaCl, 3 mM KCl, 3 mM KH_2PO_4 or 8 mM $NaHPO_4$) containing 1 % (w/v) Nonidet P40 (Bethesda Research Laboratories, Maryland, USA) to yield, after centrifugation at 10000 × *g*, a vesicle extract and pellet. The latter was extracted once with 0.52 M NaCl to yield, after centrifugation, a yolk extract and a pigment pellet. Aliquots of the cell fractions were electro-

Abbreviations. MgAc, magnesium acetate; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride.

phoresed on SDS/polyacrylamide gels [8] or were immunoprecipitated using antibodies raised against locust egg vitellin [13] or against *Xenopus* vitellogenin or yolk platelet proteins [14]. Immunoprecipitates were also analyzed on gels, which were usually fluorographed [15].

Locust egg vitellin [13] and *Xenopus* serum vitellogenin [16] were purified and used both as competitor species and as gel markers. *Xenopus* vitellogenin, prepared by either the dimethylformamide method of Ansari et al. [16] or, where indicated, the selective precipitation method of Wiley et al. [17] and iodinated using chloramine T [18], was used at a specific activity of about 0.5 mCi/mg for experiments on protein uptake by cultured oocytes. DEAE-cellulose was washed with modified Barth X pH 7.6 and where stated was added to the oocyte incubation medium. Total and poly(A)-rich RNA were prepared from the livers of normal and estrogen-treated male frogs [8] and were dissolved in 5 mM Tris pH 7.6 prior to injection. Locust fat body poly(A)-rich RNA was prepared by the method of Applebaum et al. [19].

RESULTS

The Distribution in Normal and Defolliculated Oocytes of Vitellogenin and Yolk Proteins Made under the Direction of Injected Messenger RNA

Oocytes were injected with locust fat body or *Xenopus* liver RNA and were left overnight before labelling with [^{35}S]methionine. Uninjected control oocytes were treated similarly. 24 h later the medium was collected and the oocytes were fractionated into cytosol, vesicle extract, yolk extract and pigment pellet. Samples of the medium and the various fractions were analyzed on SDS/polyacrylamide gels. In certain experiments, aliquots of cell fractions were immunoprecipitated using antibodies raised against either *Xenopus* vitelloenin or locust egg vitellin.

Manually isolated single oocytes are surrounded [20] by a closely applied layer of follicle cells, by theca cells and by an outer epithelial layer, all of which can be removed by treatment with collagenase. Oocytes lacking these layers of surrounding cells were used to evaluate the role played by the theca and follicle cells in the export and import of proteins. Furthermore, because of their increased accessibility, defolliculated oocytes were also used to investigate the pathways followed by locust and frog vitellogenins made in *Xenopus* oocytes.

Fig. 1 shows that defolliculated oocytes injected with *Xenopus* liver RNA (from estrogen-induced males) but not uninjected control oocytes (tracks 2 and 6) synthesize a protein (approximate M_r 210000) that is present in both the incubation medium (track 5) and the vesicle extract (track 1) but is virtually absent from the cytosol (data not shown). Experiments with folliculated (normal) oocytes yield the same result. The identity of the vitellogenin-like species was confirmed using specific antibodies (Figs. 3, tracks 7 and 8) and by its absence from the medium surrounding oocytes injected with liver RNA prepared from uninduced male frogs [21]. Whether defolliculated or normal oocytes are used, in many experiments only small amounts, as judged by gel analysis, of the estrogen-induced heterologous protein are found outside the oocyte: indeed in studies involving long (>48 h) labelling periods, vitellogenin usually cannot be detected either inside or outside the cell, although several other liver proteins, including albumin (identified from the results of previous immunoprecipitation studies [21]) can still be seen in the incubation medium. The yolk extract from RNA-injected defolliculated

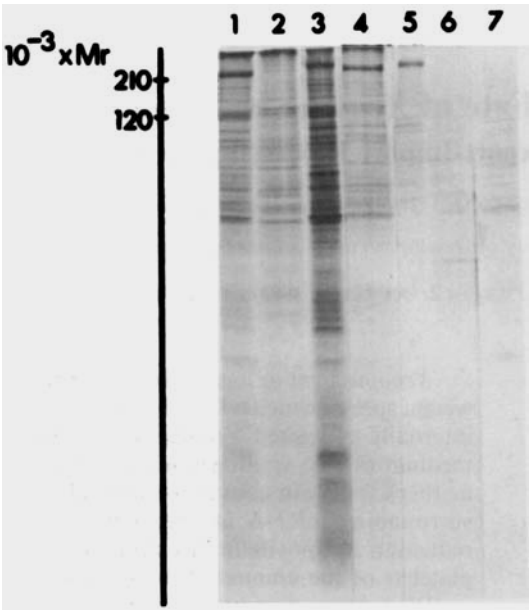

Fig. 1. *The sequestration and export of* Xenopus *and locust vitellogenins made in oocytes under the direction of injected messenger RNA.* After injection with locust fat body poly(A)-rich RNA (1 mg/ml) or *Xenopus* liver total RNA (4 mg/ml), oocytes were incubated overnight before labelling with [^{35}S]methionine for 48 h. The surrounding medium was then collected, cell fractions were prepared and analyzed on gels. Thus tracks 1–4 show vesicle extracts from oocytes injected with *Xenopus* RNA (track 1), uninjected controls (track 2), cells injected with locust RNA (track 3) and oocytes injected with *Xenopus* RNA and treated with colchicine (2 mg/ml) plus cytocholasin (25 μg/ml) (track 4). Tracks 5–7 show incubation medium surrounding oocytes injected with *Xenopus* RNA (track 5), that from uninjected controls (track 6) and that (track 7) from cells injected with *Xenopus* RNA and treated with colchicine (2 mg/ml) plus cytocholasin (25 μg/ml). With the exception of track 3, all lanes show material from defolliculated oocytes. The mobilities of *Xenopus* vitellogenin (M_r 210000) and lipovitellin (M_r 120000) markers shown

oocytes (Fig. 2, track 6) but not that from control defolliculated or normal oocytes (Fig. 2, track 5) contains radioactive lipovitellin. As shown in Fig. 3, track 1, intact vitellogenin is seen in immunoprecipitates of the yolk extract when short labelling periods are used. Radioactive lipovitellin and traces of radioactive phosvitin accumulate when the oocytes are chased with unlabelled methionine (tracks 2 and 3). Thus it is clear that the sequestration, export and processing of *Xenopus* vitellogenin made from injected messenger RNA does not require the closely applied layer of follicle cells, nor are these processes dependent upon the outer theca and epithelial layers. Furthermore, the presence of intact vitellogenin molecules in the yolk extract implies that the platelets are the site of at least some lipovitellin formation.

The nature and distribution of the products made in oocytes under the direction of locust fat body RNA is rather different, although the vesicle extract also contains a high-molecular-weight species, a doublet, which runs (Fig. 1, track 3) just behind frog vitellogenin, that is in the molecular weight range expected of the locust yolk precursor. The detailed work of Chen [22], which includes peptide mapping, has already established that a doublet arises from the translation in oocytes of two distinct locust vitellogenin messenger RNAs. As shown in Fig. 4, neither of these large polypeptides can be detected in the yolk platelet fraction (tracks 6–8), nor can they be seen in the cytosol, nor are they found in the surrounding medium (tracks 1–5). Instead the incubation

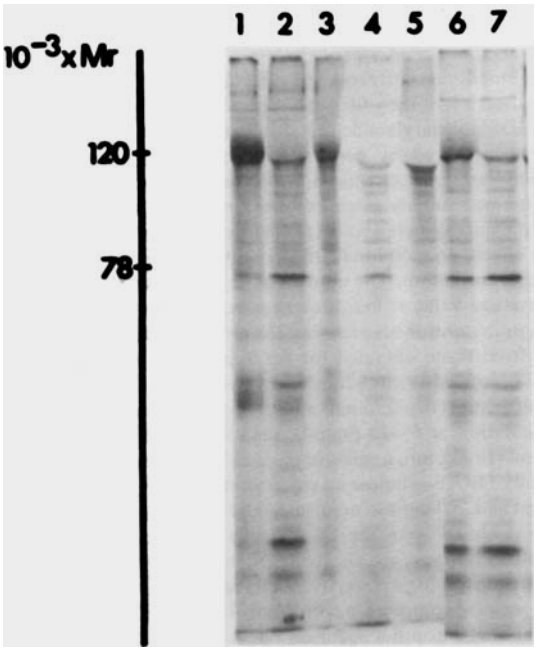

Fig. 2. *The effects of anti-vitellogenin antibodies, trypan blue, tunicamycin and defolliculation on the messenger-directed formation of platelet lipovitellin.* Oocytes were injected with *Xenopus* liver total RNA and were then incubated overnight before labelling for 48 h with [^{35}S]methionine in the presence of various reagents. Tracks 1 – 4 show RNA-injected folliculated oocytes. Thus track 1 depicts a yolk extract from oocytes injected with RNA, track 2 shows the influence of externally applied anti-vitellogenin antibody (fractionated with ammonium sulfate) on such an extract, track 3 that of externally applied trypan blue (100μg/ml) and track 4 that of tunicamycin (20 μg/ml). Track 5 shows an extract from uninjected, folliculated oocytes. Track 6 shows an extract from defolliculated, RNA-injected cells, whilst track 7 shows the effect of externally applied anti-vitellogenin antibody on such an extract from defolliculated oocytes. The mobility of marker *Xenopus* lipovitellin (M_r 120000) is shown

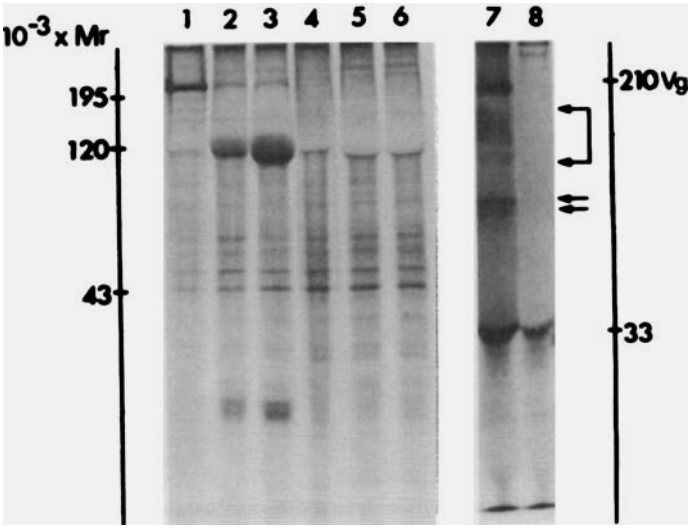

Fig. 3. *The association with yolk platelets of* Xenopus *vitellogenin made under the direction of injected messenger RNA.* Injected oocytes were labelled with [^{35}S]methionine for 5 h. Yolk platelet proteins were prepared from some of the cells, whilst the others were transferred to unlabelled medium to which nonradioactive methionine (10 mM) was added 2 h later: platelet proteins were again prepared at intervals after the start of the chase. Tracks 1 – 3 show anti-vitellogenin immunoprecipitates of yolk extracts from RNA-injected oocytes, whilst tracks 3 – 6 show immunoprecipitates from control oocytes. The pulse-chase times are as follows: tracks 1 and 4, 5-h pulse; tracks 2 and 5, 22-h chase; tracks 3 and 6, 40-h chase. Constant amounts of acid-precipitable radioactivity (material from 0.7 – 1.1 oocytes) were applied to each gel slot. Comparison with molecular weight markers revealed heterologous species in the vitellogenin (M_r 210000), lipovitellin (M_r 120000) and phosvitin (M_r 30000 – 35000) regions of the gel. In a separate experiment anti-vitellogenin antibodies were used to identify the species (M_r 210000) found in the medium surrounding liver-RNA-injected oocytes (track 7) but not control oocytes (track 8). The arrows denote the mobilities of groups of breakdown products characteristic of aged preparations of purified vitellogenin

medium from RNA-injected oocytes contains four major and several minor exported proteins (Fig. 4, tracks 1 and 2) that react with anti-(locust vitellin) antibodies (track 9 and [21]). These four major species migrate with four of the major polypeptides present [23] in locust vitellin. They are not abundant within the oocyte (tracks 10 and 11; [22]) and in particular are not detectable in the yolk fraction (tracks 6 – 8): indeed, the platelets contain no heterologous species precipitable by the rabbit antiserum which is also active [24, 19] against locust vitellogenin (data not shown). A pulse-chase experiment (Fig. 4, tracks 1 – 4) demonstrated that the locust vitellins are rapidly secreted. The export half-life is defined as the the time taken for half of the total secreted protein to accumulate in the incubation medium, and densitometer measurements of the data shown in Fig. 4 reveal that the major locust vitellogenins all have similar export half-lives of between 18 h and 25 h. Even after the two-day labelling period and the long chase there is no indication of locust vitellins or vitellogenins within the yolk platelet fraction (track 7).

The Effects of Externally Applied Anti-Vitellogenin on the Conversion of Internally Generated Xenopus *Vitellogenin to Yolk Platelet Proteins*

We wanted to find an agent that, when present in the medium surrounding an injected oocyte, would trap or destroy vitellogenin but would not itself either enter the oocyte or influence it adversely. We have tested several agents, including DEAE-cellulose, trypan blue [25], trypsin and chymotrypsin, ConA-Sepharose, an excess of uninjected oocytes (of distinctive size and pigmentation), and serum fractions containing anti-vitellogenin antibodies. All caused a reduction (see Table 1 and text) in the formation of lipovitellin, although the effect of trypan blue was rather slight (Fig. 2, track 3). The results shown are based on densitometry of the lipovitellin band; equal amounts of acid-insoluble radioactivity were applied to each gel slot thereby correcting for differences in methionine incorporation. The effect on overall oocyte protein synthesis of these externally applied agents was usually slight (Table 1) except perhaps when proteolytic enzymes were added.

The presence of *Xenopus* anti-vitellogenin antiserum in the medium surrounding the oocyte caused no significant inhibition (12 ± 13 %) of protein synthesis, and the effect of antibodies on lipovitellin formation was therefore studied in detail. Thus Fig. 2 and Table 1 show that amounts of antiserum which cause no reduction of [^{35}S]methionine incorporation nonetheless reduce radioactive lipovitellin formation by as much as 80 % (average reduction 64 ± 15 %). Since externally applied proteins penetrate the oocyte in only trace amounts [6], one can argue that an externally acting agent is reducing the conversion of internally generated vitellogenin. Furthermore, during a 48-h incubation *in vitro*, less than 1.2 % of a preparation of radioiodinated antiserum entered the oocyte,

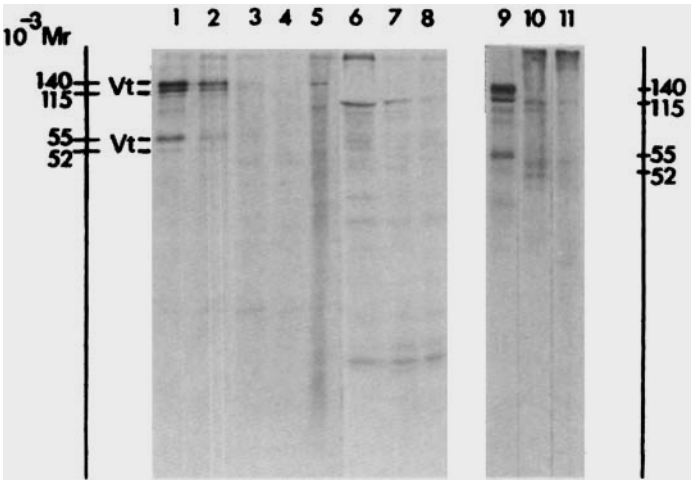

Fig. 4. *Newly-made proteins from the incubation medium and yolk platelets of oocytes injected with locust vitellogenin messenger RNA.* Oocytes were injected with poly(A)-rich locust fat body RNA (1 mg/ml), left overnight and then incubated for 48 h with [^{35}S]methionine. The surrounding medium was collected and a salt extract of platelet proteins was prepared from selected unmottled oocytes. The remaining healthy oocytes were then incubated in saline containing unlabelled methionine (10 mM) for a further 26 h, after which time the surrounding medium was collected, frozen and replaced with similar, fresh medium. The chase was continued and cell fractions were analyzed at various times. In addition, anti-(locust vitellin) immunoprecipitates of cell fractions were analyzed on a separate gel (tracks 9–11). In a further separate study, incubation medium was subjected to electrophoresis alongside purified locust egg yolk vitellins. The mobilities of the major heterologous proteins exported and the major locust vitellins were found to match each other perfectly, and the appropriate mobilities [23] have been assigned. Tracks 1–5 show incubation medium whilst tracks 6–8 show platelet protein extracts. Material from RNA-injected oocytes is labelled (+) whilst that from controls is denoted (−). Track 1 (+), 0–45-h [^{35}S]methionine labelling; track 2 (+), 26-h chase; track 3 (+), 26–52-h chase; track 4 (+), 52–75-h chase; track 5 (−), 0–48-h labelling; track 6 (+), 0–48-h labelling; track 7 (+), 0–75-h chase; track 8 (−), 0–48-h labelling; track 9 (+), immunoprecipitate of incubation medium; track 10 (+), immunoprecipitate of cytosol, and track 11 (+), immunoprecipitate of vesicle extract. Tracks 9–11 are from a different gel.

during which time the antiserum remained an effective inhibitor of lipovitellin formation (Fig. 5, track 10).

It is by no means established that the inhibitory effect of ammonium-sulfate-fractionated anti-vitellogenin antiserum is a consequence of any specific affinity for vitellogenin. Indeed unfractionated anti-albumin serum also caused an inhibition, albeit a smaller one (Fig. 5, track 3). However, as shown in Fig. 6, tracks 7–9, fractionated anti-albumin serum hardly diminishes lipovitellin formation, which suggests that the effect of the fractionated anti-vitellogenin serum is specific.

Given that it is difficult to find intact frog vitellogenin outside antibody-treated oocytes, it seemed possible that extracellular proteases [26] might be responsible for the effect on lipovitellin formation. The protective action of the serine protease inhibitor PMSF was therefore studied. However, PMSF neither stabilizes vitellogenin outside the oocyte nor inhibits the action of the antibody (Fig. 5, tracks 2 and 3). Thus, the results of our experiments with anti-vitellogenin antisera indicate strongly that an obligatory external step is involved in messenger-directed lipovitellin formation, but our studies only suggest that anti-vitellogenin antibodies are the external agents which actually block conversion of the precursor.

Table 1. *The effect of externally applied agents on messenger-directed lipovitellin formation in oocytes*

RNA-injected and control oocytes were labelled with [^{35}S]methionine prior to the extraction of the yolk platelet proteins which were then analyzed on 12.5% SDS/polyacrylamide gels. Radioactive *Xenopus* lipovitellin formation was measured by computer-aided integral densitometery of gel autoradiograms, measurements throughout the broad band of M_r 120000 being summed. In any given experiment, equal amounts of acid-precipitable radioactivity were applied to each gel slot, thereby correcting for non-specific effects on methionine incorporation when measuring radioactive lipovitellin formation. The number of separate observations contributing to an average value is indicated in parentheses. Radioactive lipovitellin formation in control oocytes was always low and these values were subtracted from those obtained for the appropriate RNA-injected oocytes. Externally applied agents whose effects were measured include anti-vitellogenin antibodies (two different antisera, one of which was also tested after radioiodination at 5–10 mg/ml), tunicamycin (20 μg/ml), anti-albumin antiserum (10 mg/ml), a mixture of colchicine (2 mg/ml) and cytocholasin (25 μg/ml), DEAE-cellulose an equal mixture of trypsin and chymotrypsin (50 μg/ml and 200 μg/ml), uninjected and folliculated oocytes and (vitellogenin was eluted from the DEAE-cellulose granules and identified by SDS/polyacrylamide gel electrophoresis), uninjected defolliculated oocytes. Acid-insoluble radioactivity was measured both inside and outside treated oocytes: comparison with control oocytes permits measurement of the effect of the external agent on oocyte protein synthesis

Additions to medium surrounding mRNA-injected oocytes	Reduction (increase) in				Oocyte morphology
	Xenopus lipovitellin formation after correction for any inhibition of protein synthesis		oocyte protein synthesis		
	%	(number of observations)			
Anti-vitellogenin antiserum	71	(1)	(11)		normal
Anti-vitellogenin antiserum (ammonium-sulfate-fractionated)	64±15	(5)	12±13		normal
Anti-albumin antiserum	40	(1)	15		normal
Anti-albumin antiserum (ammonium-sulfate-fractionated)	10	(1)	15		normal
Tunicamycin	97± 2	(5)	39±18		normal
Colchicine plus cytocholasin	93± 3	(2)	40± 4		nucleus displaced pigment disrupted
DEAE-cellulose	55± 1	(2)	15		normal
Trypsin plus chymotrypsin (50 μg/ml)	25	(1)	20		follicular layers
(200 μg/ml)	95	(1)	39		loosened

The injection of total *Xenopus* liver RNA into *Xenopus* oocytes often leads to the formation of a platelet protein whose electrophoretic mobility (approximate M_r 150000) matches neither that of vitellogenin nor its major cleavage products. The polypeptide, designated X, can be seen clearly in Fig. 5 (tracks 1–4, 8 and 10) and is absent from extracts of platelets from control oocytes (tracks 5, 6, 7 and 9). We have not ruled out the possibility that exported liver proteins, other than vitellogenin, are imported by oocytes.

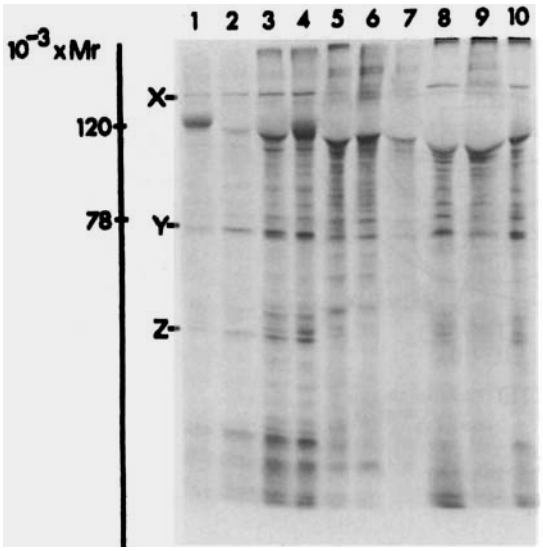

Fig. 5. *The effects of anti-(*Xenopus *vitellogenin) and anti-albumin antibodies, protease inhibitors, iodinated antibodies and tunicamycin on lipovitellin formation.* Yolk extracts were prepared from RNA-injected and control oocytes which had been treated with a variety of agents whilst incubating with [^{35}S]methionine (48 h). Tracks 1–4, 8 and 10 show gel analysis of yolk extracts from RNA-injected oocytes, as indicated by the symbol (+), whilst tracks 5–7 and 9 display material from uninjected control cells, as indicated by the symbol (−). Additions to the surrounding incubation medium are as follows: tracks 1 (+) and 6 (−), no additions; tracks 2 (+) and 5 (−), PMSF-trated anti-vitellogenin antiserum (fractionated); tracks 3 (+) and 7 (−), PMSF-treated anti-albumin antiserum (unfractionated); track 4 (+), nonradioactive vitellogenin (degraded, as judged by gel electrophoresis); tracks 8 (+) and (−), tunicamycin and track 10, ^{125}I-labelled anti-vitellogenin antiserum. Treatment with protease inhibitor involved addition of 0.12 mM PMSF to both antibody stock solutions and to the antibody-containing medium at the start of the incubation. Molecular weight markers of M_r 78000 and M_r 120000 (lipovitellin) are shown

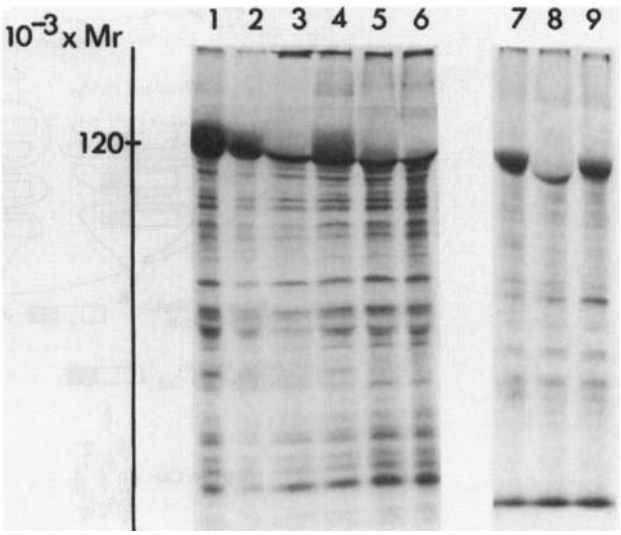

Fig. 6. *The coincubation of mRNA-injected defolliculated oocytes and small defolliculated oocytes.* Defolliculated cells of two size classes were isolated. The large oocytes (diameter *circa* 1.0 mm) were injected with poly(A)-rich *Xenopus* liver RNA (3 mg/ml) and were left overnight after which intact cells were selected for coincubation with small oocytes (0.5 mm). The number of oocytes in each size class was found to be the same at the end as at the beginning of the 48-h incubation in [^{35}S]methionine. The large and small oocytes were then fractionated, separately, into cytosol, vesicle extract and yolk extract. Gel analysis of the latter is shown as follows: the symbol (+) denotes proteins from mRNA-injected cells, whilst the symbol (−) indicates that material was obtained from uninjected oocytes. Track 1 (+), large oocytes; track 2 (+), large oocytes separated, after coincubation, from an equal number (12) of small oocytes; track 3 (−), 12 small oocytes separated, after incubation, from 12 large RNA-injected cells; track 4 (+), 12 large oocytes separated after coincubation from 24 small oocytes; track 5 (−), 24 small oocytes separated from 12 large RNA-injected cells; track 6 (−), 12 large uninjected oocytes. Tracks 7–9 show a separate experiment in which the effects of ammonium-sulfate-fractionated antisera on mRNA-directed platelet lipovitellin formation were compared: thus track 7 shows yolk platelet proteins from oocytes injected with vitellogenin mRNA; track 8 reveals the effect of externally applied anti-vitellogenin antiserum on such oocytes whilst track 9 shows the effect of external anti-albumin antiserum

The Coincubation of mRNA-Injected and Uninjected Scavenger Oocytes

If mRNA-directed lipovitellin formation involves an obligatory external step, uninjected oocytes present in the same incubation vessel might also convert internally generated vitellogenin to platelet lipovitellin. Initial experiments using folliculated oocytes gave little indication that uninjected oocytes could use yolk precursors made by RNA-injected oocytes. It seemed that the cells secreting vitellogenin were topologically favoured with regard to recapture of the precursor protein. However, we noticed that vitellogenin often accumulates in relatively large amounts (Fig. 1, track 5) in the medium surrounding RNA-injected defolliculated oocytes. In the hope that topological constraints would be reduced in such cells, mRNA-injected and defolliculated oocytes were incubated together: small vitellogenic oocytes were used as the feeder cells, and were counted at the beginning and end of the experiment. The large, RNA-injected oocytes were also counted. A 24-h wound-healing period ensured a low level of leakage [21]. Fig. 6 shows yolk extracts from large and small oocytes which were separated from each other after being incubated together: with a twofold excess of small cells, some radioactive lipovitellin accumulates in the yolk platelets of the feeder oocytes. Densitometry reveals a level 30% above the background level present in uninjected oocytes incubated alone. There is a corresponding decrease (of 35%) in lipovitellin formation within the cells containing heterologous messenger. Thus the feeding experiments provide important independent evidence favouring the export-import model.

The Effect of Tunicamycin, Colchicine and Cytocholasin on Messenger-RNA-Directed Platelet-Protein Formation in Oocytes

The role played by N-linked oligosaccharides can be studied using tunicamycin, which is known to block the *N*-glycosylation, but not the export of several proteins from oocytes [27]. Fig. 5 (tracks 8 and 9) show that tunicamycin treatment (20 μg/ml) of oocytes making frog vitellogenin blocks, almost completely (97±2%, Table 1), the formation of radioactive lipovitellin. Injected tunicamycin (20 μg/ml approximate internal concentration) is also effective.

Colchicine alone, and especially in combination with cytocholasin, blocks the export of several heterologous proteins [28]: if this effect on secretion is general, the action of these drugs provides a further test of the export/import model. As in the tunicamycin study, we have corrected for any effect on methionine incorporation by applying equal amounts of acid-precipitable radioactivity/gel slot. Thus Fig. 1 (track 4) shows that a mixture of the drugs does not block vitellogenin

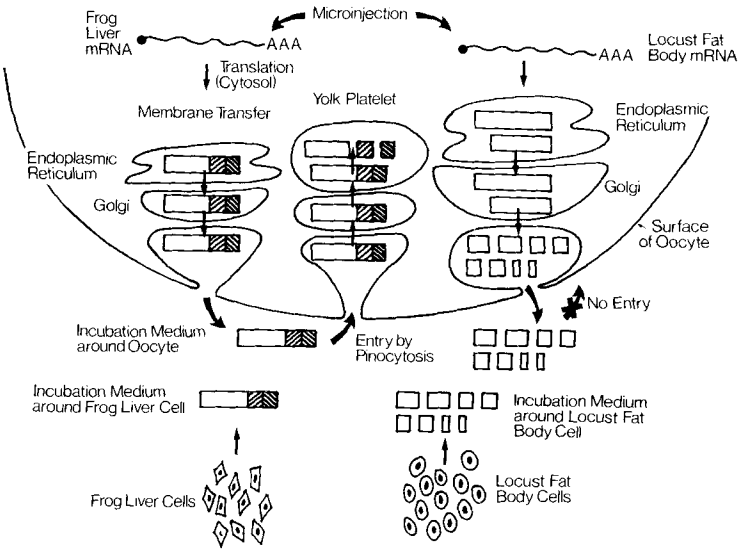

Fig. 7. *The fate of* Xenopus *and locust vitellogenins made in* Xenopus *oocytes: an export-import processing model.* Microinjection of locust fat body or *Xenopus* liver messenger RNA leads to the synthesis and sequestration within membranes of high-molecular-weight vitellogenin species (see Fig. 1). There is no direct evidence that the heterologous proteins then progress through the Golgi and secretory vesicles, although such structures are present in oocytes: there is, however, direct evidence that some at least of the vitellogenin is exported, the locust protein probably being processed just prior to secretion (see Fig. 1 and 4). The pinocytotic uptake pathway shown is based on the work of others [33]. The occurrence of intact frog vitellogenin in washed platelets (see Fig. 3) is consistent with the internally generated precursor adopting the pathway followed by exogenous frog vitellogenin [9, 32]. The four major locust polypeptides exported from oocytes programmed with fat body RNA migrate with the four locust vitellins. We have not established that processing of the minor species is correct, although minor polypeptides are seen in the expected molecular weight range. Nonetheless, as judged by SDS/polyacrylamide gel electrophoresis, protein-processing pathways in oocytes are characteristic of the cell used to prepare the donor RNA. The model is consistent (see Fig. 2, 5 and 6) with the effects of a variety of agents added to the medium surrounding RNA-injected oocytes. It must be stressed that such experiments do not prove the export-import processing model nor do they exclude the existence of purely internal pathways of messenger-directed lipovitellin formation

synthesis, but does cause a near complete inhibition ($93 \pm 3\%$, Table 1) of messenger-directed lipovitellin formation. Vitellogenin cannot be detected in the medium surrounding the drug-treated RNA-injected oocytes. Thus the studies with colchicine and cytocholasin provide additional indirect evidence that vitellogenin must be exported before it can be converted to platelet lipovitellin.

DISCUSSION

The translational and post-translational systems of the frog oocyte are neither species-specific nor cell-type-specific, yet are highly selective. In general proteins made in oocytes by messenger RNAs from terminally differentiated cells are modified [29], separated into compartments [3] and exported [4, 21] just as they are in the parent cell types. There are only a few exceptions to this rule [12, 21, 30–35]. It seems that the nature and extent of processing is determined by the amino acid sequence of the polypeptide chain and is seldom restricted by the spectrum of enzymes present in the oocyte. The mobilities on SDS/polyacrylamide gels of the proteins made in oocytes under the direction of *Xenopus* liver or locust fat body mRNA support these generalizations. The initial products sequestered within oocyte vesicles are similar to those found within hepatocytes or fat body cells and the major protein species exported by oocytes are similar, immunologically and electrophoretically, to the major polypeptides secreted by the insect or amphibian cells [5, 9, 10, 23, 36–44].

The conversion of internally generated *Xenopus* vitellogenin to platelet lipovitellin is reduced somewhat (data not shown) by the presence of vitellogenin in the incubation medium surrounding the oocyte, which suggests there is overlap between the pathways for conversion of exogenous and endogenous yolk protein precursors. The addition of an IgG fraction containing anti-vitellogenin antibodies to the medium abolishes messenger-directed lipovitellin formation without affecting significantly the overall rate of protein synthesis. The oocyte is only slightly permeable to proteins in general [6] and immunoglobulins in particular and it is therefore unlikely that the action of the anti-vitellogenin is internal. Furthermore, iodinated proteins other than vitellogenin are degraded rapidly following endocytosis [45]. Moreover, the inhibitory action of the amonium-sulfate-fractionated antiserum (IgG fraction) towards messenger-directed lipovitellin formation is probably caused by a specific macromolecular component (i.e. anti-vitellogenin) because fractionated anti-albumin antiserum has little effect.

The influence of small defolliculated oocytes on lipovitellin formation within RNA-injected oocytes provides independent evidence that externally acting scavengers can reduce conversion of the precursor protein. In these experiments, some at least of the trapped vitellogenin is not destroyed completely, because radioactive lipovitellin accumulates within the platelets of the uninjected feeder oocytes (see Fig. 6). Thus the results obtained with externally applied agents are consistent with an export/import pathway, but until trapping of secreted vitellogenin can be correlated quantitatively with reduced lipovitellin formation, proof of such a pathway (particularly that it is the only route) is lacking. Our studies with colchicine and cytocholasin, known inhibitors of the export of heterologous secretory proteins from oocytes, are also merely compatible with a pathway having an obligatory external step. The model (see Fig. 7) is nonetheless an attractive one, for it reconciles the vitellogenin mRNA and protein injection experiments [7, 8] and the export of heterologous secretory proteins [4, 21] with the classical studies of vitellogenin uptake by pinocytosis [6, 9]. The accumulation of locust vitellogenins in the incubation medium and not in any subcellular com-

partment supports the idea that all stable vitellogenin molecules are exported and is consistent with the above model. The failure to import internally generated locust vitellogenins and convert them to stable platelet proteins reveals, somewhat surprisingly [46, 47], a degree of species specificity in the pathway.

What role, if any, do the surrounding follicular layers [20, 48] play in messenger-directed lipovitellin formation? Defolliculated oocytes can still export vitellogenin and will convert it to platelet proteins [49], despite some accumulation of the precursor in the incubation medium. The role played by N-linked oligosaccharides was investigated using tunicamycin, which blocks *N*-glycosylation of proteins in oocytes [27]. The drug causes a dramatic (see Table 1) inhibition of lipovitellin accumulation. An effect on vitellogenin stability, export [50] or even synthesis [51] is possible, but unlikely [27]. It is also possible that oocytes cannot import the kind of vitellogenin exported by tunicamycin-treated cells. Such an explanation would implicate N-linked oligosaccharides in the highly specific recognition processes involved in vitellogenin uptake. Although the oocyte is an excellent system for investigating such topological determinants, the present paper merely explains in broad terms how it is that vitellogenin molecules originating in different compartments can reach the same final destination.

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REFERENCES

1. Mertz, J. E. & Gurdon, J. B. (1977) *Proc. Natl Acad. Sci. USA, 74*, 1502–1506.
2. Lane, C. D., Marbaix, G. & Gurdon, J. B. (1971) *J. Mol. Biol. 61*, 73–91.
3. Zehavi-Willner, T. & Lane, C. D. (1977) *Cell, 11*, 683–693.
4. Colman, A. & Morser, J. (1979) *Cell, 17*, 517–526.
5. Wallace, R. A. & Dumont, J. N. (1968) *J. Cell Physiol. 72*, 73–89.
6. Wallace, R. A. & Jared, D. W. (1976) *J. Cell Biol. 69*, 345–351.
7. Dehn, P. F. & Wallace, R. A. (1973) *J. Cell Biol. 58*, 721–724.
8. Berridge, M. V. & Lane, C. D. (1976) *Cell, 8*, 283–297.
9. Jared, D. W., Dumont, J. N. & Wallace, R. A. (1973) *Dev. Biol. 35*, 19–28.
10. Brummett, A. R. & Dumont, J. N. (1977) *Dev. Biol. 60*, 482–486.
11. Ford, C. C. & Gurdon, J. B. (1977) *J. Embryol. Exp. Morph. 37*, 203–209.
12. Lane, C. D., Shannon, S. & Craig, R. (1979) *Eur. J. Biochem. 101*, 485–495.
13. Harry, P., Pines, M. & Applebaum, S. W. (1979) *Comp. Biochem. Physiol. 63B*, 287–293.
14. Berridge, M. V., Farmer, S. R., Green, C. D., Henshaw, E. C. & Tata, J. R. (1976) *Eur. J. Biochem. 62*, 161–171.
15. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem. 46*, 83–88.
16. Ansari, A. Q., Dolphin, P. J., Lazier, C. B., Munday, K. A. & Akhtar, M. (1971) *Biochem. J. 122*, 107–113.
17. Wiley, H. S., Opresko, L. & Wallace, K. A. (1979) *Anal. Biochem. 97*, 145–152.
18. Hunter, W. M. & Greenwood, F. C. (1962) *Nature (Lond.) 194*, 495–496.
19. Applebaum, S. W., James, T. C., Wreschner, D. H. & Tata, J. R. (1981) *Biochem. J. 193*, 209–216.
20. Dumont, J. N. & Brummett, R. (1978) *J. Morphol. 155*, 73–98.
21. Lane, C. D., Colman, A., Mohun, T., Morser, J., Champion, J., Kourides, I., Craig, R., Higgins, S., James, T. C., Applebaum, S. W., Ohlsson, R. I., Paucha, E., Houghton, M., Matthews, J. & Miflin, B. J. (1981) *Eur. J. Biochem. 111*, 225–235.
22. Chen, T. T. (1980) *Arch. Biochem. Biophys. 201*, 266–276.
23. Chen, T. T., Strahlendorf, P. W. & Wyatt, G. R. (1978) *J. Biol. Chem. 253*, 5325–5331.
24. Gellisen, G., Wajc, E., Cohen, E., Emmerich, H., Applebaum, S. W. & Flossendorf, T. (1976) *J. Comp. Physiol. B, 108*, 287–301.
25. Wallace, R. A. & Ho, T. (1972) *J. Exp. Zool. 181*, 303–318.
26. Soreq, H. & Miskin, R. (1981) *FEBS Lett. 128*, 305–310.
27. Colman, A., Lane, C. D., Craig, R., Boulton, A., Mohun, T. & Morser, J. (1981) *Eur. J. Biochem. 113*, 339–348.
28. Colman, A., Morser, J., Lane, C. D., Besley, J., Wylie, C. & Valle, G. (1981) *J. Cell Biol. 91*, 770–780.
29. Asselbergs, F. A. M. (1979) *Mol. Biol. Reports, 5*, 199–208.
30. Kindas-Mugge, I., Lane, C. D. & Kreil, G. (1974) *J. Mol. Biol. 87*, 451–462.
31. Lane, C. D., Champion, J., Haiml, L. & Kreil, G. (1981) *Eur. J. Biochem. 113*, 273–281.
32. Rapoport, T. A., Thiele, B. J., Prehen, S., Marbaix, G., Cleuter, Y., Hubert, E. & Huez, G. (1978) *Eur. J. Biochem. 87*, 229–233.
33. Vassart, G., Refetoff, S., Brocas, H., Dinsart, C. & Dumont, J. E. (1975) *Proc. Natl Acad. Sci. USA, 72*, 3839–3843.
34. Labarca, C. & Paigen, K. (1977) *Proc. Natl Acad. Sci. USA, 74*, 4462–4465.
35. Asselbergs, F. A. M., Koopmans, M., Venrooij, W. J. van & Bloemendal, H. (1979) *Exp. Eye Res. 28*, 475–482.
36. Wallace, R. A. & Jared, D. W. (1969) *Dev. Biol. 19*, 498–526.
37. Redshaw, M. R. & Follett, B. K. (1971) *Biochem. J. 124*, 759–766.
38. Opresko, L., Wiley, H. S. & Wallace, R. A. (1980a) *Cell, 22*, 47–57.
39. Bergink, E. W. & Wallace, R. A. (1974) *J. Biol. Chem. 249*, 2897–2903.
40. Wallace, R. A. (1978) in *Vertebrate Ovary* (Jones, R. E., ed.) pp. 469–502, Plenum Publishing, New York.
41. Tata, J. R. & Smith, D. F. (1979) *Rec. Progr. Hormone Res. 35*, 47–95.
42. Wahli, W., Dawid, I. B., Ryffel, G. U. & Weber, R. (1981) *Science (Wash. DC) 212*, 298–304.
43. Wyatt, G. R. & Pan, M. L. (1978) *Annu. Rev. Biochem. 47*, 779–817.
44. Engelman, F. (1979) *Adv. Insect Physiol. 14*, 49–108.
45. Opresko, L., Wiley, H. S. & Wallace, R. A. (1980b) *Proc. Natl Acad. Sci. USA, 77*, 1556–1560.
46. Wallace, R. A., Deufel, R. A. & Misulovin, Z. (1980) *Comp. Biochem. Physiol. 65B*, 151–155.
47. Kunkel, J. G. & Pan, M. L. (1976) *J. Insect Physiol. 22*, 809–818.
48. Browne, C. L., Wiley, H. S. & Dumont, J. N. (1979) *Science (Wash. DC) 203*, 182–183.
49. Wallace, R. A., Ho, T., Salter, D. W. & Jared, D. W. (1973) *Exp. Cell Res. 82*, 287–295.
50. Leavitt, R., Schlesinger, S. & Kornfeld, S. (1977) *J. Biol. Chem. 252*, 9018–9023.
51. Seagar, M. J., Miquelis, R. D. & Simon, C. (1980) *Eur. J. Biochem. 113*, 91–96.

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