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# Non-parallel Kinetics and the Role of Tissue-specific Factors in the Secretion of Chicken Ovalbumin and Lysozyme from *Xenopus* Oocytes

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# Non-parallel Kinetics and the Role of Tissue-specific Factors in the Secretion of Chicken Ovalbumin and Lysozyme from *Xenopus* Oocytes

D. Cutler, C. Lane† and A. Colman

*Department of Biological Sciences*
*University of Warwick, Coventry, CV4 7AL, England*

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Ovalbumin and lysozyme made in *Xenopus* oocytes under the direction of injected chicken oviduct messenger RNA accumulate at different rates in the surrounding culture medium. Pulse-chase experiments confirm that the intrinsic rate of lysozyme secretion from oocytes is 12 times that of ovalbumin. This slower rate of ovalbumin export is maintained following injection of either diluted oviduct RNA or purified ovalbumin messenger, the latter having been obtained by hybridization to cloned ovalbumin complementary DNA. These results suggest that the differential rates of transport observed in oocytes are not the consequence of competition for amphibian or avian factors and show that oviduct-specific proteins are not required for ovalbumin secretion.

## 1. Introduction

The route followed by secretory proteins as they progress from their intracellular site of synthesis to the extracellular medium is now well charted, mainly as a result of detailed studies of the pancreatic acinar cell (Palade, 1975). However, knowledge of the molecular mechanisms involved in protein translocation is far from complete. More is known about events prior to entry into the Golgi apparatus than about subsequent processes. Thus the signal hypothesis (Blobel & Sabatini, 1971; Milstein *et al.*, 1972; Blobel & Dobberstein, 1975*a*,*b*) is well established (Blobel *et al.*, 1979) and there is now evidence that luminal proteins are shuttled in clathrin-coated vesicles from the endoplasmic reticulum to the Golgi apparatus (Rothman & Fine, 1980). In contrast, little is known about events within the Golgi complex or about post-Golgi movement. This bias reflects, in part, the lack of suitable *in vitro* systems for studying the later steps in the secretory pathway.

How are proteins directed to different subcellular destinations? The sorting mechanism is at present unknown, and it is not even clear whether all the signals involved are intrinsic to the protein itself, although the concept of specific topogenic sequences within proteins has been advanced (Blobel, 1980). There are also unresolved questions associated with the secretory process as a whole, one of

† Present address: National Institute of Medical Research, Mill Hill, London NW7 1AA, England.

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which is whether all proteins exhibit parallel kinetics of secretion. Such kinetics have been observed in the pancreatic acinar cell where diverse secretory proteins are discharged from pancreatic cells in fixed ratios which reflect the heterogeneous composition of the individual secretory vesicles (Kraehenbuhl *et al.*, 1977), which in turn reflects the relative rates of intracellular synthesis and degradation of the different secretory proteins. Reports of non-parallel release from pancreatic cells (Rothman, 1975,1977) and some other systems (Smilowitz, 1980; Gianattasio *et al.*, 1980) are unconvincing, but non-parallel secretion has recently been demonstrated in rat hepatoma cells (Strous & Lodish, 1980). An approach we have taken to this question involves the use of *Xenopus laevis* oocytes as a surrogate system to study the export of many different secretory proteins.

In the oocyte, almost without exception heterologous secretory proteins synthesized under the direction of micro-injected messenger RNAs are both modified correctly and exported (Colman & Morser, 1979; Lane *et al.*, 1980). With certain proteins (e.g. guinea pig mammary gland (Colman & Morser, 1979) and chick oviduct (Colman *et al.*, 1981) proteins) the accumulation of the various species in the surrounding medium does not reflect their intracellular concentrations. In this paper we demonstrate for the chick oviduct proteins ovalbumin and lysozyme that this disproportionality is a consequence of their non-parallel secretion from oocytes. Furthermore, using purified ovalbumin messenger RNA we show that no other oviduct-specific proteins are required for ovalbumin export, nor even is the rate of ovalbumin secretion influenced by the presence of other chicken-specific proteins.

## 2. Material and Methods

### (a) *Preparation of mRNA*

Polyadenylated oviduct mRNA was prepared from laying Rhode Island Red hens (Palmiter, 1973). Ovalbumin mRNA was purified by hybridization to cloned ovalbumin complementary DNA (pOv230) bound to diazobenzyloxymethyl (DBM) paper (Alwine *et al.*, 1977) followed by elution with formamide buffer (Smith *et al.*, 1979). pOv230 DNA (McReynolds *et al.*, 1977) was a kind gift from M. Wickens.

### (b) *Handling of oocytes*

Oocytes were prepared, micro-injected and labelled in modified Barths solution containing [$^{35}$S]methionine (Amersham; 1 mCi/ml, 50 Ci/mmol) as previously described (Colman & Morser, 1979). At various times groups of oocytes and incubation media were removed from culture for assay and stored at $-70$°C.

### (c) *Gel electrophoresis*

Homogenized oocytes and incubation media were either diluted with an equal volume of sample buffer (20% glycerol, 2% sodium dodecyl sulphate, 1% $\beta$-mercaptoethanol, 1 mM-phenylmethylsulphonyl fluoride, 0·01% bromophenol blue in 20 mM-Tris·HCl (pH 7·6)) or first immunoprecipitated (see below). Portions equivalent to either 0·125 oocytes or the incubation medium surrounding 2 oocytes were electrophoresed on 12·5% (w/v) or exponential 10% to 22·5% polyacrylamide gels (Laemmli, 1970). Gels were then fixed and either directly exposed to X-ray film (Fuji photo film Co. Ltd.) or fluorographed (Bonner &

Laskey, 1974). Two-dimensional electrophoresis was performed as described by Colman *et al.* (1981) modified here by the use of gradient gels for the 2nd dimension.

(d) *Immunoprecipitation*

Portions (50 μl) of homogenized oocytes or incubation media were diluted with 450 μl of detergent mix containing 100 mM-Tris·HCl (pH 8·2), 100 mM-KCl, 5 mM-$MgCl_2$, 1% Triton X-100, 0·5% sodium dodecyl sulphate, 1% sodium deoxycholate. 5 μl of anti-ovalbumin or anti-lysozyme antibody were then added and the resulting mixture incubated for 1 h at 4°C before the addition of 50 μl of formalin-fixed *Staphylococcus aureus* envelopes (Kessler, 1975). After an overnight incubation the bacterial envelopes were pelleted, washed 3 times in detergent mix, and the bound protein eluted by heating (100°C for 2 min) in sample buffer. The anti-ovalbumin antibody used in experiments illustrated in Figure 7 was a kind gift from M. Houghton. Other anti-ovalbumin antibody was purchased from Miles Biochemicals. Anti-lysozyme antibody was a kind gift from A. Sippel.

(e) *Microdensitometry*

Microdensitometry was performed using a Joyce-Loebl double-beam scanning microdensitometer (model 3CS). Quantitative fluorographs of polyacrylamide gels were prepared according to the methods of Laskey & Mills (1975).

## 3. Results

### (a) *Ovalbumin and lysozyme are secreted at different rates*

The oocyte programmed with chicken oviduct mRNAs has proved a useful system with which to study the secretory process (Colman *et al.*, 1981). Oocytes microinjected with oviduct mRNA synthesize and secrete significant amounts of both chick ovalbumin and lysozyme (Lane *et al.*, 1980; Colman *et al.*, 1981). At least six different modified ovalbumin polypeptides are made in the oocyte, these resolving as three or two bands on gradient or single percentage polyacrylamide gels, respectively (Colman *et al.*, 1981).

Analysis of ovalbumin and lysozyme export from oocytes reveals (Fig. 1) that as much radioactive lysozyme as ovalbumin accumulates in the incubation medium although net ovalbumin synthesis is much greater. The simplest interpretation of this observation is that lysozyme is secreted faster than ovalbumin. However, alternative explanations are possible. For example, this apparent difference in ratios would occur if lysozyme synthesis was substantially curtailed during the labelling period as a result of the preferential degradation of the lysozyme messenger RNA. This possibility is excluded by the observation (Fig. 1) that the disproportionality in the relative rates of accumulation persists 96 hours after mRNA injection. These data also establish the stability within oocytes of both these secretory protein mRNAs.

Alternatively, differential accumulation in the medium of the two chicken proteins could be due to transitory changes in their individual rates of synthesis during the 24 hour labelling period. We have therefore studied the synthesis and export of these two proteins throughout a 24 hour labelling period. Under the experimental conditions used, incorporation of [$^{35}$S]methionine into protein remains linear for at least 24 hours (data not shown). The results shown in Figure 2

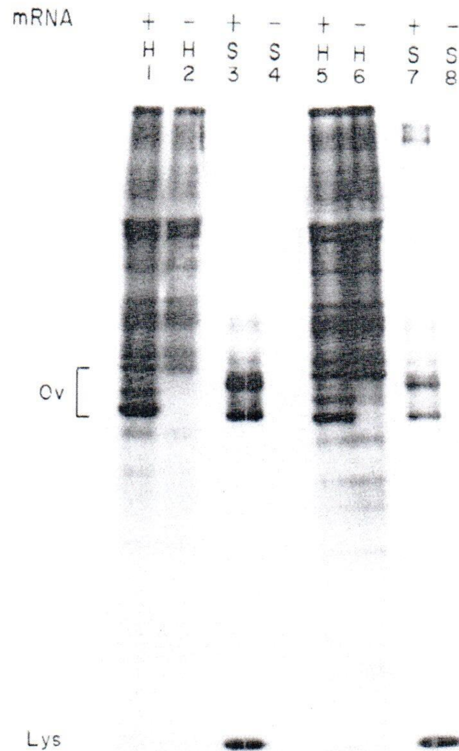
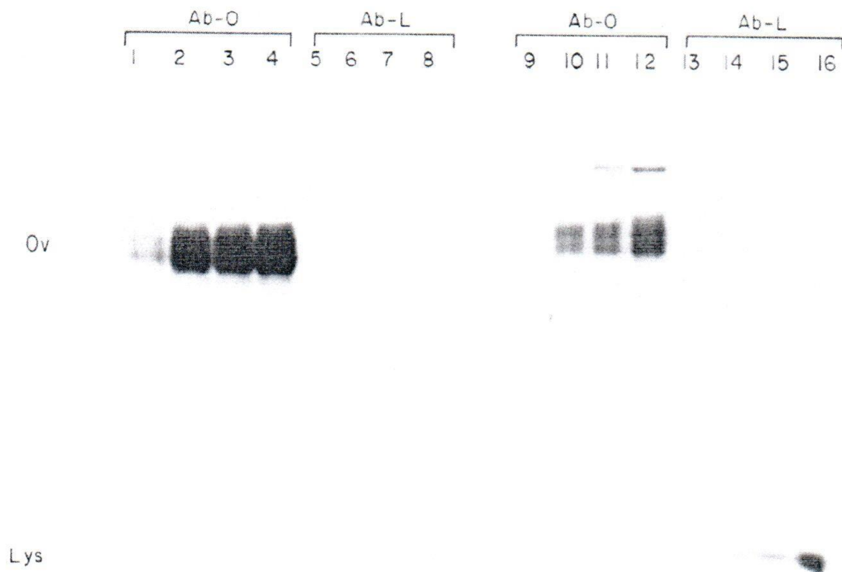


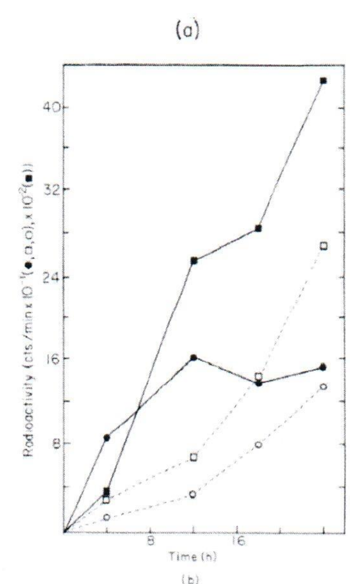

FIG. 1. Secretion of chick proteins by oocytes. Oocytes were injected with 30 nl of oviduct mRNA (1 mg/ml in distilled water) and cultured in modified Barths saline for 24 h (tracks 1 to 4) or 72 h (tracks 5 to 8). Healthy oocytes were transferred in groups of 5 to 30 $\mu$l of saline containing [$^{35}$S]methionine (1 mCi/ml, 50 Ci/mmol) and the incubation was continued for a further 24 h. Groups of 10 oocytes were homogenized and the homogenates (H) were prepared for electrophoresis: incubation media (S) were prepared directly for electrophoresis. Samples were electrophoresed on 10% to 22·5% exponential gradient polyacrylamide gels, which were then fixed and fluorographed. The symbol Ov. denotes the position of immunoprecipitable ovalbumin. The symbol Lys. refers to the position of marker lysozyme and immunoprecipitable lysozyme.

reveal that an approximately 12-fold disproportionality in the net synthesis and secretion of these two proteins is maintained during continuous labelling.

The above results might be reconciled with models of secretion based on parallel discharge if, in the oocyte, all the intracellular lysozyme but only a small proportion of the intracellular ovalbumin was available for export. This would occur if most of the intracellular ovalbumin, which is normally located within a membraneous compartment (Colman *et al.*, 1981), becomes trapped in a storage or other non-secretory membrane bound compartment. Under these circumstances the ratio between secreted and secretable protein could be similar for both ovalbumin and lysozyme. To test this possibility pulse-chase experiments were performed. Injected oocytes were labelled by incubation for 24 hours in medium

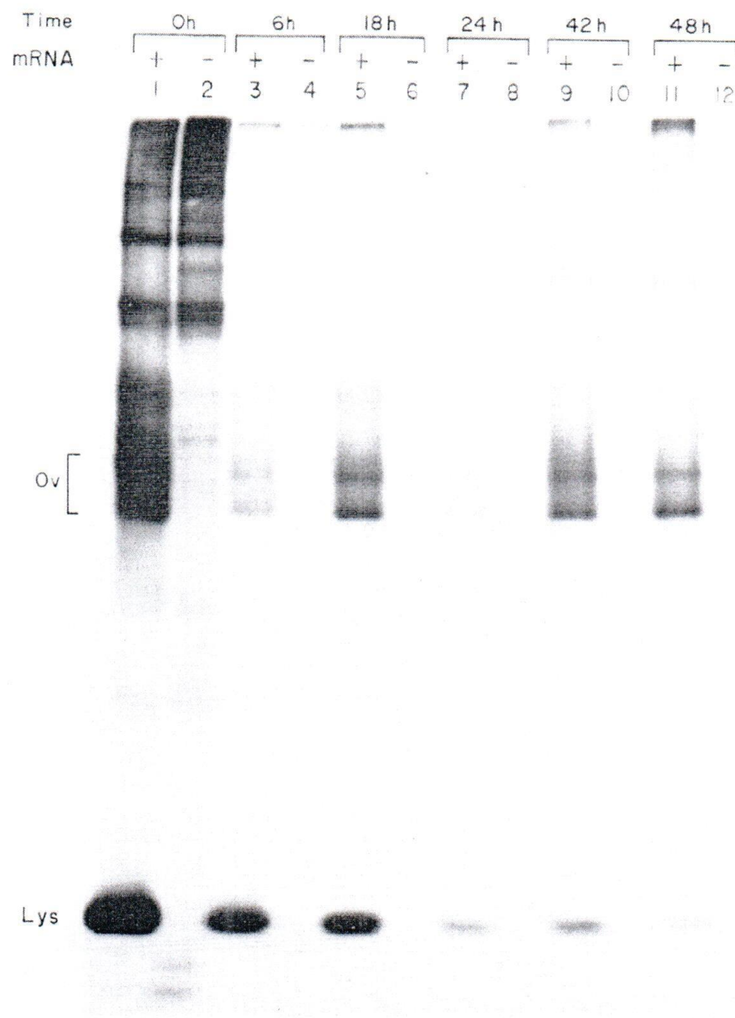



(a)

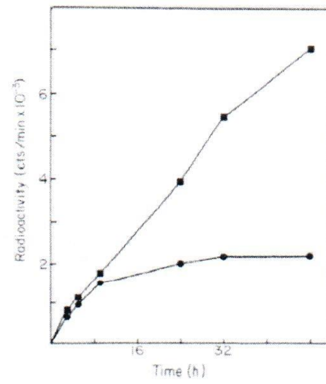



(b)

FIG. 2. Kinetics of chick protein secretion. (a) Oocytes were injected with 30 nl oviduct mRNA (1 mg/ml) and cultured in unlabelled saline (24 h) followed by a further period of up to 24 h in labelled saline (see Fig. 1). Oocytes (1 to 8) and incubation media (9 to 16) removed at various times, were immunoprecipitated and electrophoresed on a 12·5% gel. Tracks 1, 5, 9, 13: 0 to 4 h; tracks 2, 6, 10, 14: 0 to 12 h; tracks 3, 7, 11, 15: 0 to 18 h; tracks 4, 8, 12, 16: 0 to 24 h. Ab-O, anti-ovalbumin antibody. Ab-L, anti-lysozyme antibody. (b) The regions of a gel (a) corresponding to the positions of immunoprecipitated ovalbumin and lysozyme were excised and counted in an NCS (Amersham/Searle)/toluene-based scintillant. Data were corrected to show amounts per oocyte. Ovalbumin: intracellular —■——■—; secreted - - - □ - - - □ - - -. Lysozyme: intracellular —●——●—; secreted - - - ○ - - - ○ - - -.




(a)




(b)

FIG. 3.

TABLE 1

*Secretion during pulse-chase experiments*

| | Chase (h) | Oocyte Cts/min × $10^{-3}$ /oocyte | Oocyte Total loss cts/min × $10^{-3}$ /oocyte | Medium Cts/min × $10^{-3}$ /oocyte | Medium Recovery† (%) |
|---|---|---|---|---|---|
| Ovalbumin | 0 | 93·5 | 38·9 | 41·6 | 106 |
| | 72 | 54·6 | | | |
| Lysozyme | 0 | 6·1 | 3·5 | 3·3 | 94 |
| | 72 | 2·6 | | | |

Pulse-chase experiment performed as described in the legend to Fig. 3. Quantitative immunoprecipiation of samples with anti-ovalbumin or anti-lysozyme antibodies was performed. Portions of immunoprecipitates were then precipitated with trichloroacetic acid and counted. Results are from one typical experiment.

† The amount recovered in the medium is expressed as a percentage of the amount lost from the oocyte.

containing [$^{35}$S]methionine and then transferred to medium containing 10 mM-unlabelled methionine. Under these conditions no further incorporation of [$^{35}$S] methionine into protein occurs (Lane *et al.*, 1979). The medium surrounding these "chased" oocytes was collected for analysis at intervals over the following 48 hours. As can be seen from Figure 3(a), the changing ratio of ovalbumin to lysozyme in the incubation medium shows that during the chase period, most of the secreted lysozyme appears in the medium before a small fraction of the ovalbumin has been secreted. By excision and quantitation of the protein bands from the gel it can be shown clearly that the rate of extracellular accumulation of ovalbumin relative to its synthesis is slower than that for lysozyme (Fig. 3(b)). The accumulation of lysozyme ceases after about 32 hours, when all the potentially secretable protein has been exported. Surprisingly, a substantial proportion (approx. 40%) of the intracellular lysozyme is retained within the oocyte (Table 1) where it is present within the vesicle fraction of the oocyte (data not shown).

FIG. 3. Pulse-chase of oocytes making chicken lysozyme and ovalbumin. (a) Oocytes were injected with oviduct mRNA (1 mg/ml) and cultured first in unlabelled Barths saline and then for 24 h in labelled saline (see Fig. 1). The surrounding incubation media were then stored at −70°C (0 h). The remaining oocytes were then incubated in groups of 5 each in 100 μl of saline containing 10 mM-unlabelled methionine. The media surrounding all batches of oocytes were then removed and replaced intermittently. Track headings indicate the protein accumulated before the beginning of the chase (O), between 0 to 6 h (6 h), 6 to 18 h (18 h), 18 to 24 h (24 h), 24 to 42 h (42 h), 42 to 48 h (48 h). Secreted protein was precipitated with ice-cold trichloroacetic acid and the precipitates dissolved in sample buffer before electrophoresis on a 10% to 22·5% exponential gradient gel. Ov, ovalbumin; Lys, lysozyme. (b) Accumulation of extracellular lysozyme and ovalbumin was quantified by excision and subsequent counting of the regions of a gel (a) containing ovalbumin and lysozyme; equivalent regions of control tracks from uninjected oocytes were subtracted. Data are corrected to show amounts per oocyte. Ovalbumin: –■——■–; lysozyme: –●——●–.

In contrast the secretion of ovalbumin is still occurring at the end of the 48 hours. The time taken for half of the intracellular ovalbumin to be chased out is approximately 70 hours. All of the ovalbumin lost from the inside of the oocyte can be accounted for in the incubation medium (Table 1). The time taken for half the secretable lysozyme to be exported is approximately six hours. Thus comparison of these times indicates a 12-fold faster rate of secretion for lysozyme. Thus the possibility that the slower rate of extracellular accumulation of ovalbumin reflects a large pool of non-secretory protein is clearly not supported since over 90% of the intracellular ovalbumin would have to be sequestered in this way to account for our results. We therefore conclude that, on average, lysozyme and ovalbumin molecules move from the lumen of the endoplasmic reticulum to the exterior of the oocyte at very different rates.

(b) *Ovalbumin is modified during export*

Three electrophoretic species of ovalbumin have previously been identified on the basis of precipitation by monospecific antisera and isoelectric focusing (Colman *et al.*, 1981). It is evident from Figure 1 that intracellular and secreted ovalbumins differ in the relative abundance of the species present. Microdensitometry tracings (Fig. 4) of intracellular and secreted ovalbumin reveal that the middle band (band 2) is virtually absent from the secreted ovalbumin but strongly represented within the oocyte. The converse situation applies to the slowest migrating species (band 1). However, when the same samples are run on two-dimensional gels (Fig. 5), there is no significant difference in the distribution of radioactivity between the various polypeptides species; co-electrophoresis of the two samples indicates no species unique to either sample. The molecular basis of the one-dimensional difference is

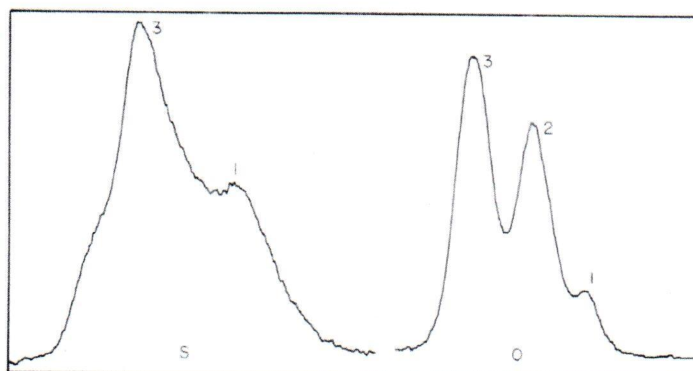



FIG. 4. Comparison of intracellular and secreted ovalbumin species. Oocytes were injected with oviduct mRNA (1 mg/ml) and cultured for 24 h in labelled saline after the usual pre-labelling incubation. Samples of intracellular (O) and secreted (S) ovalbumin were immunoprecipitated and electrophoresed on a 10% to 22·5% exponential gradient gel (c.f. Fig. 1). Quantitative fluorographs were used to prepare microdensitometry tracings. Species are labelled (1, 2, 3) in order of increasing electrophoretic mobility.

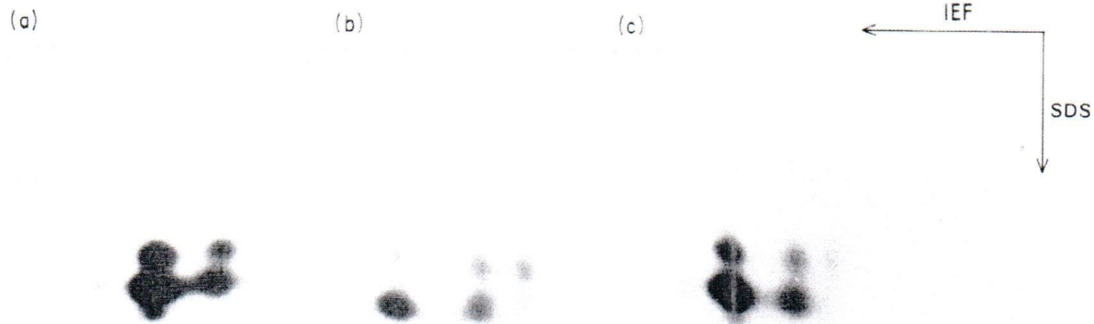



FIG. 5. Two-dimensional electrophoresis of intracellular and exported ovalbumins. Identical samples to those shown in Fig. 4 were prepared for 2-dimensional electrophoresis. Quantitative fluorographs of intracellular (a), secreted (b) and a mixture of the two (c) are shown. The mixture in (c) was designed to contain equal cts/min from both samples. IEF, isoelectric focussing. SDS, sodium dodecyl sulphate/polyacrylamide gel.

therefore not clear. However, during chase experiments the relative abundance of the ovalbumin species within the oocyte remains constant (data not shown), an observation that indicates that there is no preferential secretion of any ovalbumin polypeptide and that the putative molecular differentiation of intracellular and secreted ovalbumin occurs just prior to exocytosis.

### (c) *Ovalbumin and lysozyme do not compete for export from the oocyte*

Until recently it was not at all clear that the oocyte was a secretory cell. However, whilst the oocyte has now been shown to secrete a specific subclass of frog proteins, the level of this secretion is low when compared to the maximum rates of secretion of a wide variety of heterologous proteins (Mohun *et al.*, 1981). Can the lower relative rate of ovalbumin secretion be ascribed to its unfavourable competition with lysozyme for some limiting component of the oocyte's secretory apparatus? We have tested this hypothesis by injecting several concentrations of mRNA and assaying the ratios of intracellular to extracellular ovalbumin and lysozyme following a 24 hour incubation in medium supplemented with $[^{35}S]$-methionine. Figure 6 shows that the difference in ratios is maintained over a 20-fold variation in the concentration of mRNA injected. The range of concentrations

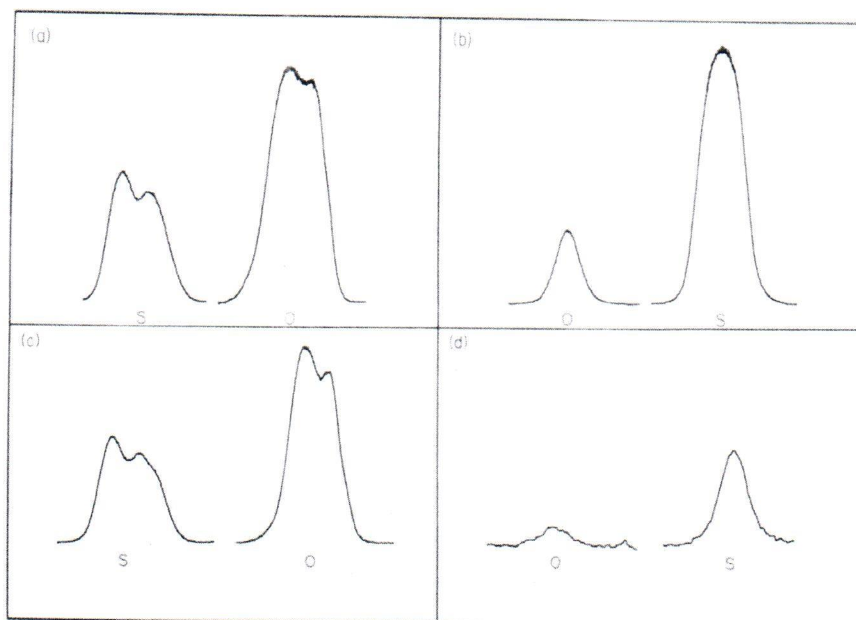



FIG. 6. Injection of different concentrations of oviduct mRNA. Oocytes were injected with mRNA at 4 mg/ml ((a) and (b)) or 0·2 mg/ml ((c) and (d)), cultured for 24 h in labelled saline and frozen, as were incubation media. Immunoprecipitates of intracellular (O) and secreted (S) ovalbumin ((a) and (c)) and lysozyme ((b) and (d)) were electrophoresed on a 12·5% gel. The gel was fixed, fluorographed, and microdensitometry tracings prepared. Different microdensitometry wedges and exposure times were used in preparing the various panels, which are therefore not directly intercomparable. However, quantitation by excision and counting of the relevant regions of the gel indicates that the 2 proteins are subject to differential *translational* control in the oocyte with the 20-fold reduction in mRNA concentration resulting in a 9·8-fold and 3·1-fold reduction in ovalbumin and lysozyme synthesis, respectively.

used was determined by the experimental system. At 120 ng per oocyte the translational capacity of these cells becomes limiting, while below 6 ng per oocyte there are difficulties with quantitative detection of the individual chick proteins. Since alteration of the amounts of the chick proteins present within the oocyte does not affect their differential relative rates of secretion we conclude that the oocyte has (for oviduct proteins) spare secretory capacity. This is not compatible with the hypothesis that ovalbumin export is being retarded by scarcity of some oocyte factor necessary for the secretion of both ovalbumin and lysozyme.

### (d) *Ovalbumin secretion is not influenced by other chicken-specific proteins*

The rate of ovalbumin secretion from oocytes injected with a diverse population of oviduct messengers could be limited by or dependent on the availability of a chicken-specific component as opposed to a frog component. Lysozyme could also compete with ovalbumin for this factor. These possibilities were investigated using

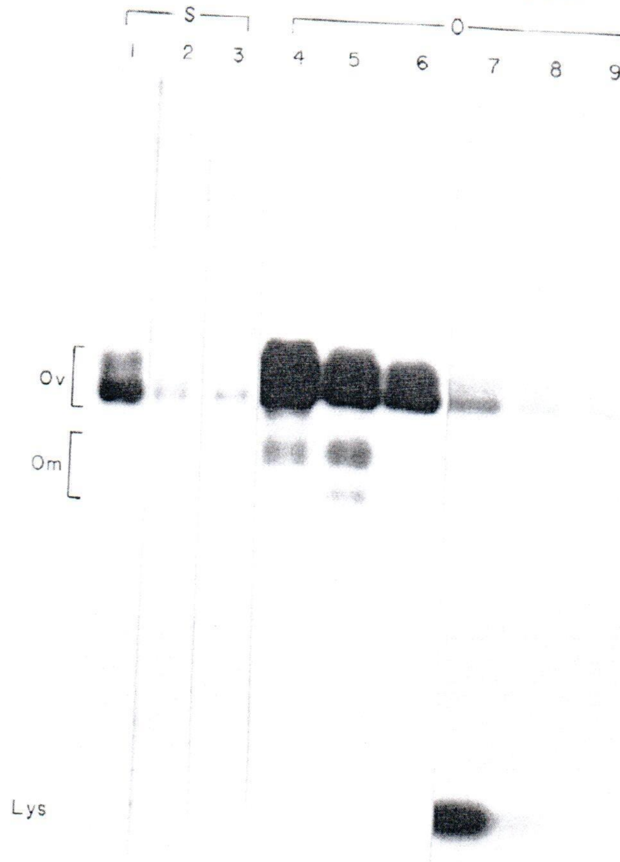



FIG. 7. Injection of purified ovalbumin mRNA. Ovalbumin mRNA was purified from oviduct mRNA by hybridization to cloned ovalbumin complementary DNA (see Materials and Methods). The residual mRNA that remained unbound during the hybridization was recovered from the reaction mixture by dilution of the latter with 2 vol. 0·3 M-sodium acetate (pH 6·8) followed by precipitation with ethanol. The yield of both bound and residual mRNA was low, due, perhaps, to degradation during hybridization. Residual mRNA, purified ovalbumin mRNA and the original oviduct mRNA preparation (1·0 mg/ml) were injected into oocytes cultured exactly as described in the legend Fig. 1. The oocytes were then processed and oocyte (O) and incubation medium (S) samples immunoprecipitated with anti-ovalbumin or anti-lysozyme antibody. The resulting immunoprecipitates were electrophoresed on $12\frac{1}{2}\%$ linear polyacrylamide gels. In order to visualize faint bands, exposures of 3 days (tracks 1 to 6) and 10 days (tracks 7 to 9) have been used. Tracks 1, 4 (0·15 vol. loaded) 7: injection of untreated mRNA. Tracks 2, 5, 8: injection of unbound mRNA. Tracks 3, 6, 9: injection of purified ovalbumin mRNA. Tracks 1 to 6 are anti-ovalbumin immunoprecipitations (this antibody contains anti-ovomucoid activity (Colman *et al.*, 1981)). Tracks 7 to 9 are anti-lysozyme immunoprecipitations. Ov, ovalbumin: Om, ovomucoid: Lys, lysozyme.

ovalbumin mRNA isolated from the crude messenger preparation by hybridization to filters containing cloned ovalbumin complementary DNA (see Materials and Methods). The purity of the ovalbumin mRNA was confirmed by the absence of chicken lysozyme and ovomucoid amongst the translation products formed in the

oocytes (Fig. 7, cf. tracks 5 and 6, 8 and 9). Both the purified mRNA and the mRNA that was not hybridized were injected into oocytes. Excess mRNA was used in the hybridization and thus the unbound mRNA population contained ovalbumin mRNA. Similar amounts of ovalbumin were made in both sets of oocytes. More importantly, similar amounts of ovalbumin were exported from both sets of oocytes, even though lysozyme was also synthesized and secreted from the oocytes injected with the residual mRNA. Consequently, in the oocyte both the export of ovalbumin and probably the rate of this process are determined by the properties of the ovalbumin molecule itself.

## 4. Discussion

Extensive studies on individual secretory proteins of the pancreatic acinar cell indicate a kinetic parallelism for both the transport and release of a majority of these proteins (Kraehenbuhl *et al.*, 1977; Tartakoff *et al.*, 1975). Such observations are consistent with the Palade model of secretion (Palade, 1975), which envisages the permanent segregation and mass translocation of secretory proteins within intracellular membranes. Rothman has cited evidence for the non-parallel discharge of pancreatic proteins, explaining this phenomenon mechanistically in terms of the unequal partition of sequestered secretory proteins within the cytosol, the cytosol being advocated as the immediate precursor pool for secretion (Rothman, 1975). This minority view has been seriously questioned, especially in the light of a series of immunocytochemical studies that have failed to demonstrate the presence of secretory proteins in the cytosol of the acinar cell (Kraehenbuhl *et al.*, 1977; Geuze *et al.*, 1979; Bendayan *et al.*, 1980). Moreover, we have shown that chicken ovalbumin miscompartmentalized in the cytosol of the *Xenopus* oocyte is never secreted, in contrast to identical molecules segregated within the endoplasmic reticulum of the frog cell (Colman *et al.*, 1981). Nonetheless, recent reports have provided evidence of different pathways and of different intrinsic rates in the export of proteins. Thus, Smilowitz (1980) showed that the appearance of the acetylcholine receptor protein on the cultured myotube surface and the release of acetylcholinesterase from the cells were differentially affected by the presence of the ionophores nigericin and monensin. Whilst the interpretation of these data has now been seriously questioned (Rotundo & Fambrough, 1980), it is also conceptually easier to envisage cellular discrimination between, as in this case, an integral membrane protein and a secretory protein, than between two secretory proteins. Recently Gianattasio *et al.* (1980) have demonstrated the differential release of various glycoproteins from bovine anterior pituitary slices. However, they recognize that since a heterologous group of cells were involved, the discharge kinetics might reflect cellular differences in the synthesis and intracellular transport of the various proteins. In contrast, Strous & Lodish (1980) have clearly demonstrated that albumin is secreted twice as fast as transferrin from rat hepatoma cells.

The data presented here show that two secretory proteins, ovalbumin and lysozyme, are exported at intrinsically different rates from a single cell, the frog

oocyte. This phenomenon does not seem likely to have a trivial explanation. For example, although ovalbumin is normally glycosylated whilst lysozyme is not, both non-glycosylated and glycosylated ovalbumin are secreted at the same rate from oocytes (Colman *et al.*, 1981). Moreover guinea pig α-lactalbumin, a non-glycosylated protein, which shows homology to chick lysozyme both in amino acid sequence and size (Brew, 1972), is secreted very slowly from oocytes (Lane *et al.*, 1980). We find the rate of ovalbumin secretion to be unchanged in the presence of either lowered (Fig. 6) or undetectable (Fig. 7) production of lysozyme. The simplest explanation of our results is that intracellular translocation of lysozyme and ovalbumin proceed at intrinsically different rates. If the pancreatic acinar cell serves as a model for the oocyte and secretory proteins are translocated from the Golgi to the extracellular space in discrete vesicles then this differential rate must reflect the relative retardation of ovalbumin during the preceding stages. Alternatively, the two proteins might be segregated in the oocyte and translocated by different routes. Although there is no evidence for this latter model we are currently investigating this possibility in the oocyte using immunocytochemical methods.

Translocation of proteins from the endoplasmic reticulum to the Golgi apparatus and from the Golgi apparatus to other sites is now thought to occur in clathrin-coated vesicles (Rothman & Fine, 1980). Destination specificity must reside both in coat component(s) and in the transported protein contents. Since oocytes correctly locate heterologous proteins (Colman & Morser, 1979; Lane *et al.*, 1980), the envisaged specificity of the vesicle component(s) must lack species specificity. As to the sorting sequences that ensure the correct vesicularization of the transported protein, it has been suggested that these reside in the protein itself or in some specific accessory protein (Blobel, 1980). Examples of the latter arguably would include human $\beta 2$ microglobulin (Ploegh *et al.*, 1979; Krangel *et al.*, 1979) or mouse egasyn (Lusis & Paigen, 1977) in their roles of locating histocompatability antigen and $\beta$-glucuronidase, respectively, within the correct cellular membrane. We show quite clearly that this specificity must, for ovalbumin, reside in its protein sequence alone.

The oocyte has now been shown to provide a surrogate system of high fidelity for the purpose of studying protein secretion (Lane *et al.*, 1980). However, it is probable that experiments with this system will serve only to define the general features of interactions between the transported proteins and the cellular transport machinery. Thus whilst it is evident that secretion of lysozyme and ovalbumin from oocytes shows non-parallel kinetics, this phenomenon does not seem to occur in the chick oviduct: in this tissue both proteins are synthesized in the same tubular gland cells (Palmiter, 1972) before their incorporation into the egg. Since the proportions of lysozyme and ovalbumin in the egg reflect their steady-state concentrations in the tubular gland cell (Palmiter, 1972), it is probable that both proteins are secreted at similar rates. We have been unable to obtain more direct evidence of parallel secretion using isolated oviduct slices due to problems of non-specific protein leakage.

We would argue that such apparent non-conformity in the oocyte's handling of the chick proteins should not invalidate this approach since in the absence of any

well-defined oocyte secretory protein the study of heterologous secretory proteins inside the oocyte serves to illustrate the types of translocational pathways available within these cells. Clearly, ovalbumin and lysozyme are secreted from oocytes at different rates. Additionally, 40% of the lysozyme cannot be chased out of oocytes, in contrast to the behaviour of other heterologous proteins (Colman & Morser, 1979). This might reflect the highly conserved nature of lysozyme and the fact that most oocyte secretory proteins are thought to be stored for later embryonic development (Zehavi-Willner & Lane, 1977); lysozyme may therefore be miscompartmentalized in error, with these oocyte proteins.

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