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The Influence of Topology and Glycosylation on the Fate of Heterologous Secretory Proteins Made in *Xenopus* Oocytes

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Secretory proteins made in *Xenopus laevis* oocytes under the direction of heterologous messenger RNA are modified, topologically segregated and exported. Thus the oocyte may serve as a useful surrogate secretory system and we have studied some of the factors governing access to the export pathway. Unglycosylated chicken ovalbumin, synthesized and trapped in the cytosol, is not secreted but glycosylated ovalbumin, found sequestered within vesicles, is exported from oocytes. However, ovalbumin, which is transferred across the endoplasmic reticulum in the presence of tunicamycin and which is indistinguishable by immunoprecipitation, by two-dimensional gel electrophoresis and by concanavalin-A–Sepharose binding from the cytosolic form, is still secreted. Guinea-pig milk proteins and human interferon are also exported from tunicamycin-treated frog cells. These observations demonstrate that access to the endoplasmic reticulum but not glycosylation is a mandatory intermediate step in secretion, and emphasize the advantages of the oocyte as a surrogate system for the study of the later events in the gene expression pathway.

Oocytes of *Xenopus laevis* will export guinea-pig caseins and human interferon encoded by micro-injected mRNAs; heterologous non-secretory proteins are not secreted [1]. Recently we have shown that secretory proteins from such diverse sources as rats, insects and plants are also selectively exported from oocytes (Lane et al., unpublished observations). These results demonstrate the possible usefulness of the oocyte as a general system for studies of protein secretion. In this paper we further examine the fidelity of protein secretion in this system by correlating the subcellular location and secondary modification of secretory proteins with their subsequent fate.

Nascent polypeptide chains of secretory proteins contain a 'signal' sequence of some 15–30 amino acids [2–5], which interacts with a putative membrane receptor. This interaction results in the vectorial discharge of nascent polypeptides into the lumen of the endoplasmic reticulum. Secondary modification (e.g. glycosylation [6], phosphorylation [6]) of protein can occur within the endoplasmic reticulum and the use of tunicamycin, an inhibitor of glycosylation [7,8] indicates that this modification may protect proteins from degradation [9], affect the rate of secretion [10,11] or alter the intracellular destination of proteins [12]. The subsequent events in secretion are less well understood but are popularly thought to involve encapsulation of secretory polypeptides within the Golgi apparatus followed by movement of the resultant secretory vesicles to the cell surface, where exocytosis occurs [6,13,14].

It is generally believed that the co-translational transfer of secretory proteins across the membranes of the endoplasmic reticulum is a mandatory step in the secretion of these proteins [3,4]; the corollary of this theory is that proteins localized in the cytosol cannot be secreted. Much of the evidence supporting these views derives from the use of cell-free translation systems supplemented with microsomal vesicles [3–5]. Such studies do not, however, formally disprove the possibility [15,16] of some post-translational transfer *in vivo* of secretory proteins through intracellular membranes. The existence of post-translational transport systems demonstrates that there is no obligatory requirement, in principle, for proteins to be made on the membranes which they are destined to cross [17–19].

In this paper we investigate the role of glycosylation in the secretion of various avian and mammalian proteins, comparing where possible our observations using the oocyte system with results obtained using the parental cells. Moreover for one protein, ovalbumin, we confirm the prevailing view that access to the lumen of endoplasmic reticulum is essential for secretion by demonstrating that ovalbumin miscompartmentalized in the cytosol is not transferred into the endoplasmic reticulum and is not secreted.

Abbreviation. $PhMeSO_2F$, phenylmethylsulfonyl fluoride.

MATERIALS AND METHODS

Animals

Adult *Xenopus laevis*, obtained from the South African Snake Farm (Fish Hoek, Cape Province, South Africa) were kept at 19 °C.

Chemicals

Except where otherwise mentioned, all chemicals were of analytical grade and were purchased from British Drug Houses Ltd (Poole, U.K.). [^{35}S]Methionine (150–300 Ci/mmol) was supplied by the Radiochemical Centre (Amersham, U.K.). Tunicamycin was a kind gift from Dr Hamill, Eli Lilley, USA.

Preparation of Messenger RNAs

Total poly(A)-containing mRNAs were isolated by the methods of Morser et al. [20] from Namalwa cells (a human lymphoblastoid line), which had been induced to produce interferon with Sendai virus. Lactating guinea-pig mammary gland mRNAs were prepared as described by Craig et al. [21]. Chick oviduct mRNA, prepared by the method of Palmiter [22], was a kind gift of Dr M. Houghton.

Preparation and Microinjection of Oocytes

Oocytes of *Xenopus laevis* were obtained and maintained in Barth's saline as previously described [1]. Oocytes were microinjected with 30-nl aliquots of mRNA with or without tunicamycin at 40 μg/ml. Injected oocytes were cultured for 24 h at 21 °C in unlabelled Barth's saline (± 2 μg/ml tunicamycin). Unhealthy oocytes were then discarded and the remaining oocytes were cultured as batches of 5 in 30 μl fresh saline, now additionally supplemented with [^{35}S]methionine at 0.75 mCi/ml in the wells of microtitre plates for a further 24 h. Oocytes and incubation media from wells containing no poor oocytes were removed for further analysis.

Subcellular Fractionation of Oocytes

Oocytes were subfractionated by a modified single-step procedure [23]. Thus groups of 20 oocytes were homogenized in 400 μl, 50 mM NaCl; 10 mM magnesium acetate, 20 mM Tris/HCl, pH 7.6 (T buffer) supplemented with 10% (w/v) sucrose, 100 mM NaCl and 1 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$) at 4 °C. 20 μl of the homogenates (H) were spun for 2 min in an Eppendorf microcentrifuge and the resulting supernatants were retained for electrophoresis. The remaining homogenates were layered on to 1 ml T buffer containing 20% (w/v) sucrose, 1 mM $PhMeSO_2F$, in 5 ml polycarbonate tubes (Measuring and Scientific Equipment, Crawley, U.K.) and spun in a 8 × 5-ml rotor at $17000 \times g_{max}$ for 30 min at 4 °C. The supernatants representing the oocyte cytosol (C) were removed and retained for electrophoresis. The pellets containing yolk and oocyte vesicular elements were further extracted with 200 μl phosphate-buffered saline 7.6 containing 1 mM $PhMeSO_2F$ and 1% NP40 followed by centrifugation at 10000 rev./min in an Eppendorf microcentrifuge at 4 °C. The supernatant (V) containing extracted vesicles was retained for electrophoresis.

Immunoprecipitation

50-μl aliquots of oocyte fractions were diluted to 500 μl by addition of immunoprecipitation buffer consisting of phosphate-buffered saline containing 0.1% NP40, 1 mM $PhMeSO_2F$, pH 7.6, 5 μl anti-ovalbumin antibody (a kind gift of Dr M. Houghton) or anti-ovomucoid antibody (a gift of Dr M. Wickens) were added and the mixture left for 1 h at room temperature. 50 μl protein-A–Sepharose (Pharmacia), pre-equilibrated in immunoprecipitation buffer, were added and the mixtures kept overnight at 4 °C. The protein-A–Sepharose pellets were collected by centrifugation, washed three times in immunoprecipitation buffer and then processed for electrophoresis as described below.

Electrophoresis

One-Dimensional Electrophoresis. To homogenate (H), cytosol (C), vesicle (V) and incubation media (I) samples, 0.2 vol. of a solution containing 60% sucrose 0.0625 M Tris + HCl pH 6.8, 10% dodecyl sulphate 5% 2-mercaptoethanol and 0.01% bromophenol blue were added and the samples heated at 100 °C for 2 min. Immunoprecipitated proteins were recovered from the protein-A–Sepharose pellets by elution with 1 ml 6% acetic acid followed by lyophilization. Lyophilized samples were then boiled for 5 min in 50 μl of the electrophoresis buffer (above). Samples were loaded onto a 10–22½% exponential gradient or 12.5% polyacrylamide gels and run using the discontinuous buffer system of Laemmli [24]. Unless otherwise indicated each of the tracks H, C and V received the equivalent of 0.125 oocyte, whilst the I tracks received the equivalent of 1 oocyte.

Two-Dimensional Electrophoresis. Selected subcellular fractions and their respective lyophilized immunoprecipitates were prepared for two-dimensional electrophoresis by a modification of the method of O'Farrell [25], as described by Ballantine et al. [26]. The pH gradients of the isoelectric focussing dimensions were pH 6.8–4.5, whilst the second dimensions consisted of 10% dodecyl sulphate/polyacrylamide slab gels. After electrophoresis for 16 h at 12 mA gels

were fixed and then fluorographed [27] for 2–4 days. Only the lower pH range of the two-dimensional gels is displayed.

Binding of Protein to Immobilized Concanavalin A

Oocyte subcellular fractions and incubation media were prepared as described above. To 20-μl aliquots of each fraction, sodium dodecyl sulphate to 0.05% (w/v) was added, and the mixture was heated at 70 °C for 2 min. After cooling, 1 ml binding buffer was added (150 mM NaCl; 0.7 mM $MgCl_2$, 1 mM dithiothreitol, 0.7 mM $MnCl_2$, 0.7 mM $CaCl_2$, 0.05% sodium dodecyl sulphate, 20 mM Tris/HCl, pH 7.5), followed by 50 μl concanavalin-A – Sepharose (Pharmacia, Ltd) pre-equilibrated with binding buffer. The slurry was left at room temperature for 15 min, frequently mixed and, after a clearing spin, the supernatant was retained as unbound material. The pellet was then washed three times in binding buffer and extracted with 3 × 200-μl binding buffer containing 0.4 M α-methyl mannoside (Sigma). The resultant supernatants were pooled and retained as bound material. Bound and unbound material was diluted fourfold with binding buffer and then precipitated by addition of ice-cold trichloroacetic acid to 10% (v/v). Precipitates were washed with acetone, air-dried, resuspended in 50 μl electrophoresis sample buffer and 25 μl aliquots run on 12.5% polyacrylamide gels. Equivalent amounts (10 μl) of original untreated samples were mixed with an equal volume of sample buffer and electrophoresed.

RESULTS

Chick Proteins are Secreted by Oocytes of Xenopus laevis

Oocytes injected with oviduct mRNA coding mainly for conalbumin, ovalbumin, ovomucoid and lysozyme, were incubated for 24 h in media containing [^{35}S]methionine. Vesicle and cytosol fractions were then prepared from these oocytes; the vesicle fraction is thought to contain the elements of the endoplasmic reticulum whilst the cytosol fraction represents the remainder of the oocyte, excluding the yolk [28]. The electrophoretic profiles of the proteins contained in these fractions and incubation media are displayed in Fig. 1. Three ovalbumin polypeptides (OV), identified by their precipitation with antibody directed against ovalbumin (Fig. 2A, track 8), were synthesised and secreted by oocytes (Fig. 1, tracks 3 and 4); only 1 or 2 bands were resolved when similar samples were electrophoresed on 12½% gels (Fig. 3, track 5). As expected, most newly synthesised ovalbumin inside the oocyte was confined to the vesicle fraction (cf. Fig. 1, tracks 2 and 3).

Chick lysozyme also accumulated in the incubation media and proportionately more was secreted than ovalbumin. Our identification of this protein as lysozyme is based on its co-migration with marker lysozyme and on the observation that treatment of oocyte homogenates with formalin-washed *Staphylococcus aureus* envelopes [29] resulted in the specific retention of only this one protein to pelleted bacterial debris (Colman and Bhamra, unpublished); presumably the specificity for this binding resides in the bacterial peptidoglycan cell wall, the normal template for lysozyme. In some experiments two vesicularized ovomucoid polypeptides, which are probably glycosylation variants (see later), were identified by immunoprecipitation with anti-ovomucoid antibody (Fig. 2B, track 4). No secreted ovomucoid was detected; however, the glycosylated ovomucoid molecule is extremely acid-soluble and much may be lost from gels during acid fixation [22]. The poorly resolved secreted protein of molecular weight 75000 is probably conalbumin.

Glycosylation of Ovalbumin Is Not Necessary for Secretion

Ovalbumin consists of a single polypeptide chain containing one oligosaccharide unit linked to an asparaginyl residue via an *N*-glycosidic bond (*N*-glycosylation [30]. It has been shown that the addition of this oligosaccharide unit to ovalbumin synthesised by oviduct explants was prevented when tunicamycin, an inhibitor of *N*-glycosylation [7, 8], was present [31]. The effect of tunicamycin on the synthesis and secretion of ovalbumin is shown in Fig. 1. Ovalbumin synthesis, subcellular distribution and secretion were not affected, although the ovalbumin polypeptides migrated at a lower apparent molecular weight (Fig. 1, tracks 7 and 8; Fig. 2A, tracks 2 and 7). This would be expected because the absence of oligosaccharide side-chains leads to the faster migration of ovalbumin on acrylamide gels [31]. The ovalbumin bands synthesised in the presence of tunicamycin correspond in apparent molecular weight to the ovalbumin polypeptides synthesised when oviduct mRNA was translated in the wheat germ cell-free translation system (Fig. 1, track 9). When the various subcellular fractions were mixed with concanavalin-A – Sepharose, only the sequestered and secreted forms of ovalbumin from non-drug-treated oocytes were bound (Fig. 4), a result which shows that only these forms of ovalbumin contain carbohydrate.

Miscompartmentalized Ovalbumin Is Not Secreted

In several batches of oocytes we have observed a faster moving species of ovalbumin, which was found exclusively in the cytosol fraction of the oocyte (Fig. 3, track 6; Lane et al. [23]). This ovalbumin polypeptide(s) like those synthesised in the presence of

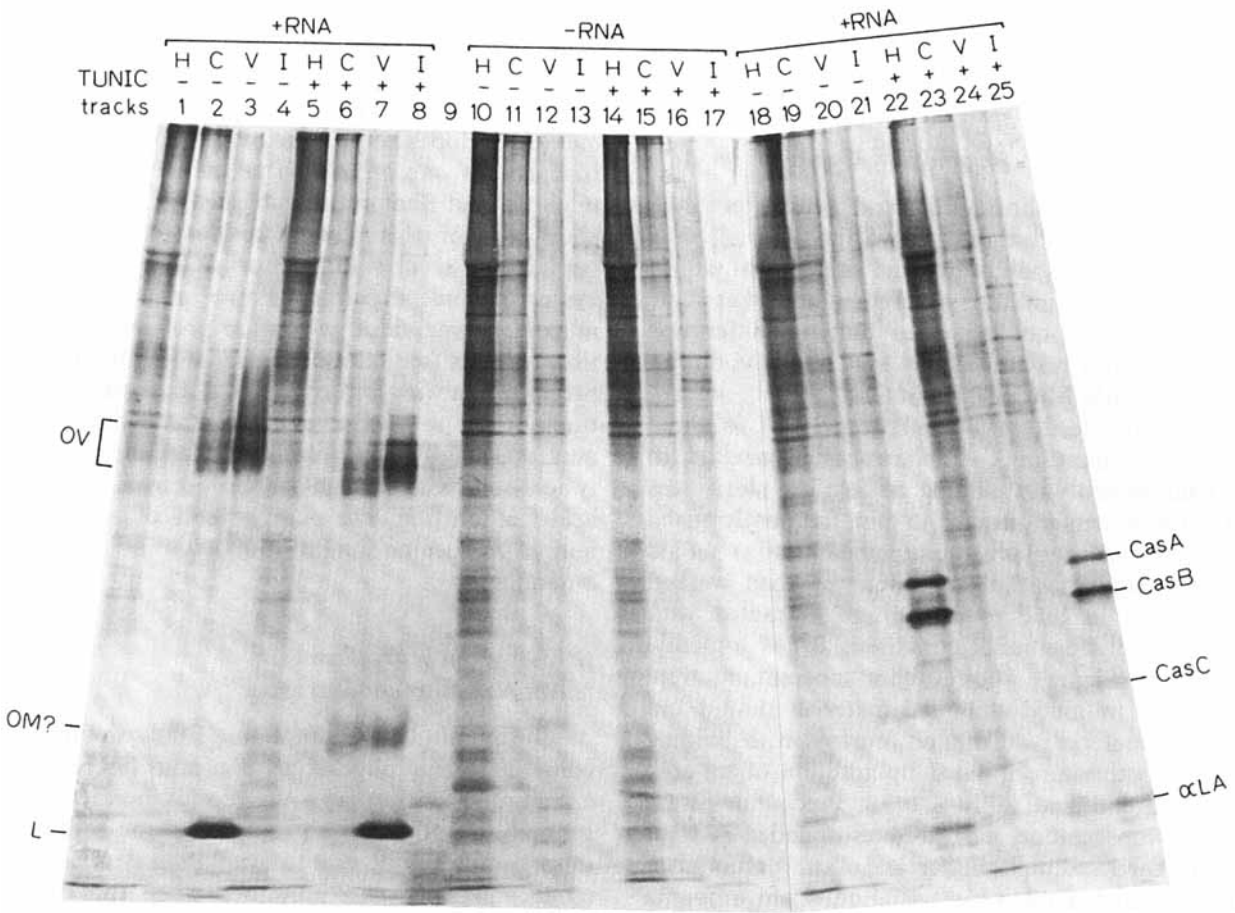

Fig. 1. *Effect of tunicamycin on protein secretion by oocytes.* Oocytes were injected with oviduct or guinea-pig mammary gland mRNAs and cultured in the presence or absence of tunicamycin, as described in Materials and Methods. At the end of the incubation oocytes and their surrounding media were prepared for electrophoresis on a $10-22^1/_2\%$ polyacrylamide gel. Each of tracks H, C and V received the equivalent of 0.125 oocyte, whilst track I received the equivalent of 1 oocyte; however, track 25 received the equivalent of only 0.5 oocyte. Tracks 1–8 show chick oviduct mRNA; track 9 contains products made in the wheat germ system {23} under the direction of oviduct mRNA; tracks 10–17 depict mock-injected controls; tracks 18–25 show mammary gland mRNA. Abbreviations used: H, oocyte homogenate; C, cytosol; V, vesicles; I, incubation medium; OV, the position of immunoprecipitable ovalbumin polypeptides; OM, position of non-glycosylated ovomucoid; L, marker lysozyme; CasA, B, C and αLA, positions of immunoprecipitable caseins A, B, C and α-lactalbumin; TUNIC, tunicamycin

tunicamycin, failed to bind to concanavalin-A–Sepharose (Fig. 4C, track 2), indicating the absence of oligosaccharide side-chains, a predictable result in view of the exclusive location of glycosylation enzymes in the endoplasmic reticulum. (The cytosolic ovalbumin bound in this assay corresponds to the glycosylated form and its presence probably results from slight breakage of vesicles during their preparation.) Furthermore both the cytosolic polypeptides and those synthesised in the presence of tunicamycin (Fig. 1) comigrate with the ovalbumin polypeptides synthesized when oviduct mRNA was translated in the wheat germ translation assay; these wheat germ products are not glycosylated (Lane et al. [23]). We conclude, therefore, that both polypeptides are similar in their apparent molecular weights and status of glycosylation. However, repeated examination of the incubation media surrounding mRNA-injected oocytes failed to reveal any trace of the cytosolic ovalbumin polypeptides (Fig. 3, track 4) although substantial secretion of 'tunicamycin' polypeptides (Fig. 1, track 8) and glycosylated ovalbumin (Fig. 1, track 4; Fig. 3, track 4) was detected.

Cytosolic and 'Tunicamycin' Ovalbumin Polypeptides Cannot Be Distinguished by Two-Dimensional Gel Electrophoresis

Although the cytosolic and 'tunicamycin' ovalbumin polypeptides both lack carbohydrate, it remained possible that they differed in the extent to which other known post-translational modifications had occurred (e.g. phosphorylation [32], N-terminal acetylation [33]). We investigated this possibility by subjecting immunoprecipitated samples containing both types of polypeptide to two-dimensional electro-

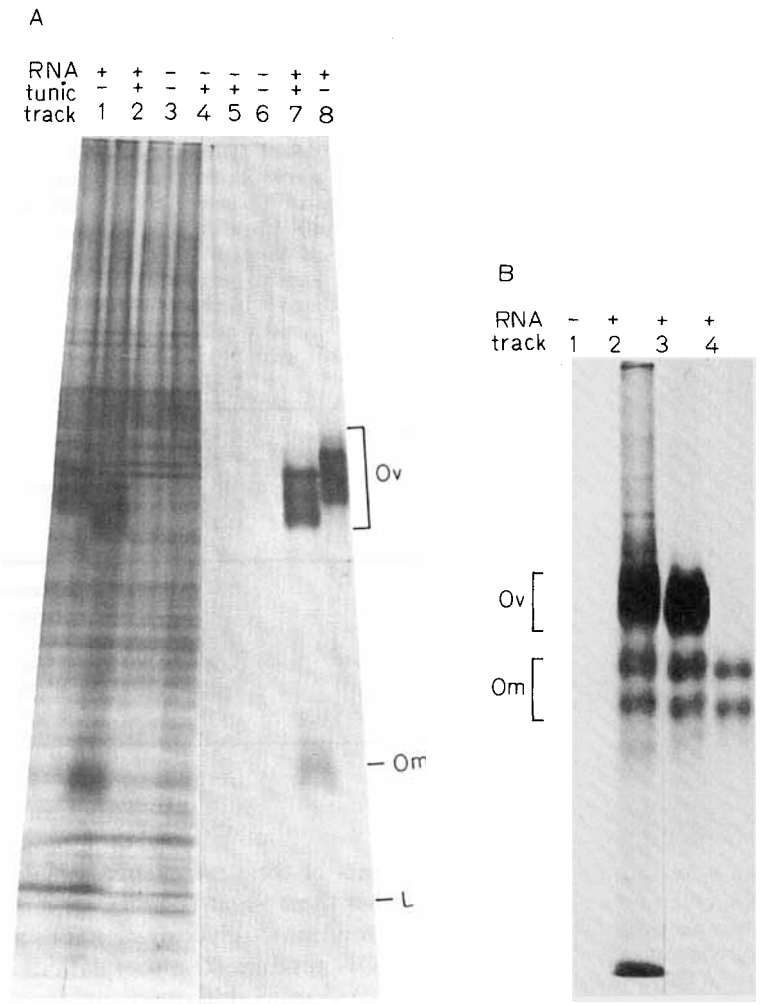

Fig. 2. *Immunoprecipitation of chick proteins made in oocytes.* Selected oocyte homogenates from the experiment shown in Fig. 1 were immunoprecipitated with either anti-ovalbumin or anti-ovomucoid antibodies and electrophoresed on 10–22½% (A) or 12½% gels (B). Only homogenates from oocytes injected with or without oviduct mRNA were sampled. (A) Tracks 1–4 oocyte homogenates; tracks 5–8 immunoprecipitated homogenates (anti-ovalbumin). (B) Track 1, immunoprecipitated oocyte homogenate (anti-ovalbumin); track 2, oocyte vesicles; the fast moving radioactive band indicates the gel front; track 3, immunoprecipitated homogenate (anti-ovalbumin); track 4, immunoprecipitated homogenate (anti-ovomucoid. Abbreviations: Ov, ovalbumin; Om, ovomucoid

phoresis [25] (Fig. 5). Comparison of the vesicularized proteins synthesized in the presence or absence of tunicamycin demonstrated in each case that six ovalbumin polypeptides could be resolved. However, those synthesized in the absence of tunicamycin were displaced to positions of higher apparent molecular weight (cf. Fig. 5a and b). Subsequent analysis of immunoprecipitated cytosolic and 'tunicamycin' polypeptides (medium and vesicle fractions) also revealed several ovalbumin polypeptides all of which were superimposable (Fig. 5d, e and f). We do not know the nature of the molecular differences which generate these species; however, they are probably post-translational modifications since four immunoprecipitable ovalbumin species have been found after microinjection of oocytes with cloned genomic ovalbumin DNA (Wickens, personal communication). Nevertheless it is clear that ovalbumin polypeptides secreted in the presence of tunicamycin have a similar molecular identity to ovalbumin polypeptides trapped in the cytosol of oocytes untreated by the drug.

We conclude from these studies that the failure of cytosolic polypeptides to be secreted is a consequence of their subcellular location, since 'tunicamycin' polypeptides, indistinguishable from untreated cytosolic polypeptides on two-dimensional gels, are secreted.

Effect of Tunicamycin on the Secretion of Other Proteins

Fig. 1 shows that chick lysozyme secretion is unaffected by tunicamycin. This was expected, since lysozyme contains no oligosaccharide side-chains. The diffuse polypeptide (apparent molecular weight

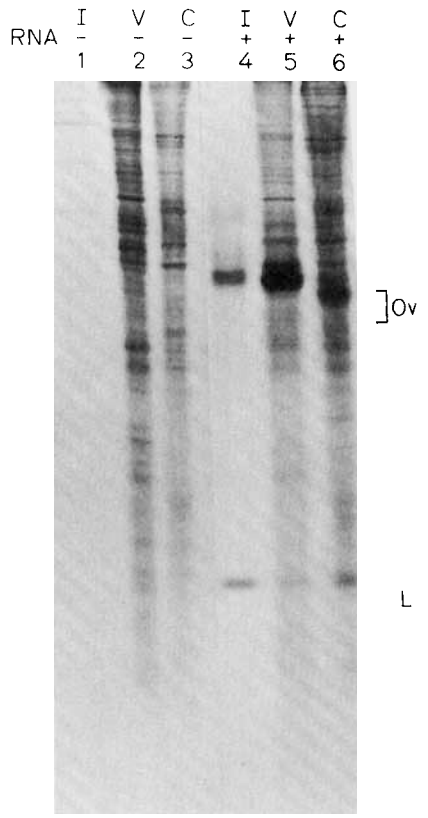

Fig. 3. *Miscompartmentation of ovalbumin in the oocyte cytosol.* Oocytes were injected with oviduct mRNA, cultured, fractionated and analysed as described in Fig. 1 the only analytical difference being electrophoresis of samples on 12.5% gels instead of 10–22.5% gradient gels. Tracks 1–3, uninjected oocytes; tracks 4–6 oviduct mRNA-injected oocytes. Abbreviations: C, cytosol; V, vesicles; I, incubation medium. No tunicamycin was used in this experiment. The postions of immunoprecipitable ovalbumin (Ov) and marker lysozyme (L) are shown

Table 1. *Effect of tunicamycin on secretion of interferon from lymphoblastoid cells and mRNA-injected oocytes*
Namalwa cells were cultured and induced as described previously [20] in the presence or absence of 2 μg/ml tunicamycin for 12 h from the time of induction. The culture media were then dialysed against phosphate-buffered saline, pH 7.6, before assay [20] on MDBK cells in which 1 unit of reference research standard (69/19) gave a titre of 0.5 unit. RNA was extracted from treated and untreated cells 9 h after induction and purified as described in Materials and Methods. 30-nl aliquots of RNA at 1 mg/ml with or without 40 μg/ml tunicamycin were injected into oocytes and the oocytes cultured in batches of 20 in 400 μl Barth X with or without 2 μg/ml tunicamycin at 21 °C. The incubation media were removed and replaced after 24 h and 48 h and assayed for interferon as above

Cells	Incubation period	Interferon in media	
		control	+ tunicamycin (2 μg/ml)
	h	reference research units/ oocyte or 10^6 cells	
Oocyte	0–24	16	16
	24–48	20	20
Namalwa cells	0–12	50000	62000

25000), observed after tunicamycin treatment, was identified as ovomucoid by specific immunoprecipitation (data not shown). This protein was also immunoprecipitated by the anti-ovalbumin antibody (Fig. 2A, tracks 2 and 7; Fig. 5d and e), which also contains a small amount of anti-ovomucoid, though no anti-conalbumin or anti-lysozyme antibodies (Colman, unpublished, and Fig. 2B, cf. tracks 3 and 4). The appearance of one ovomucoid spot after tunicamycin treatment contrasts with the two seen before (cf. Fig. 5a and b) and must reflect the existence within *oocytes of two* species of ovomucoid, glycosylated to different extents.

We have also examined the effect of tunicamycin on the secretion of the guinea-pig caseins and α-lactalbumin. It is known that guinea-pig [34] (in contrast to rat [35]) α-lactalbumin is unglycosylated. However, whilst it is clear that guinea-pig caseins A, B and C are glycosylated [36], only casein C contains significant amounts of the neutral sugars [36]. It is not known whether these sugar residues are linked to asparagine NH_2 residues (*N*-glycosylation) or serine OH or threonine OH residues (*O*-glycosylation); in other species oligosaccharide side-chains are exclusively *O*-linked [37]. Fig. 1 shows that in the presence of tunicamycin the electrophoretic positions of the secreted caseins remain unchanged, a result which argues against the presence in guinea-pig caseins of significant amounts of *N*-linked oligosaccharide. As expected tunicamycin did not influence the secretion or mobility of α-lactalbumin.

Quantification of the effects of tunicamycin on the secretion of these proteins was based on comparison of radioactivity incorporated into the various polypeptide bands. Our conclusions would, however, be questionable if tunicamycin treatment resulted in a change in the size of the oocyte's internal methionine pool. This is unlikely, since total [^{35}S]methionine incorporated into oocyte protein was unchanged. However, we have circumvented this problem, at least for one protein, by analysing the secretion of human lymphoblastoid interferon from oocytes injected with mRNA from induced human lymphoblastoid cells; in these experiments interferon was quantified by bioassay. Although lymphoblastoid interferon is *N*-glycosylated (Chadha, personal communication), the results shown in Table 1 demonstrate that the secretion of interferon from oocytes and lymphoblastoid cells was unaffected by tunicamycin.

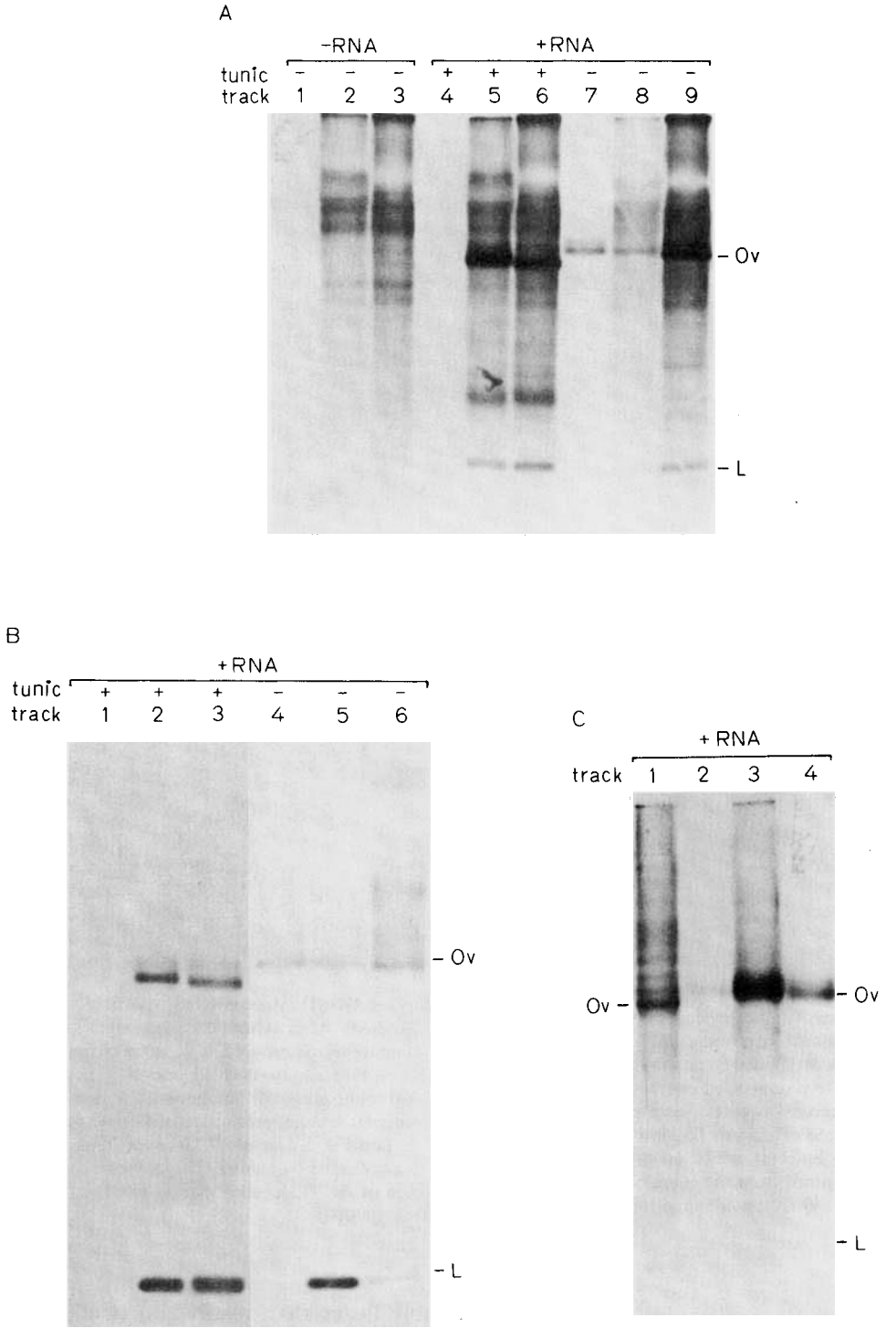

Fig. 4. *Binding of ovalbumin to concanavalin-A – Sepharose.* Subcellular fractions and incubation media were prepared from oocytes which had been injected with oviduct mRNA and cultured with or without tunicamycin. Samples were then challenged with concanavalin-A – Sepharose as described in Materials and Methods. Bound and unbound material was electrophoresed on 12.5% gels: Gels were fluorographed for 3 days except tracks 1 – 3 (B) 10 days. Comparison of the ratio of ovalbumin to lysozyme in the original and unbound fractions, especially in the secreted material (B), revealed that substantial amounts of control ovalbumin (no tunicamycin), though no tunicamycin-derived ovalbumin, were adsorbed to the concanavalin-A – Sepharose. Although recovery of the adsorbed ovalbumin was low, prolonged fluorography tracks 1 – 3 (B) failed to reveal any bound ovalbumin after tunicamycin treatment, a result consistent with the invariant ratio of ovalbumin to lysozyme in the original (track 3) and unbound (track 2) fractions. (A) Vesicle fractions: tracks 1, 4 7 bound fraction; tracks 2, 5, 8 unbound fraction; tracks 3, 6, 9 original fraction. (B) Incubation media: tracks 1, 4 bound fraction; tracks 2, 5 unbound fraction; tracks 3, 6 original fraction. Track 6 received in error, only 0.2 vol. of aliquot. (C) Vesicle and cytosol fractions: track 1, unbound cytosol fraction; track 2, bound cytosol fraction; track 3, unbound vesicle fraction; track 4, bound vesicle fraction. The positions of immunoprecipitable ovalbumin (Ov) and marker lysozyme (L) are shown

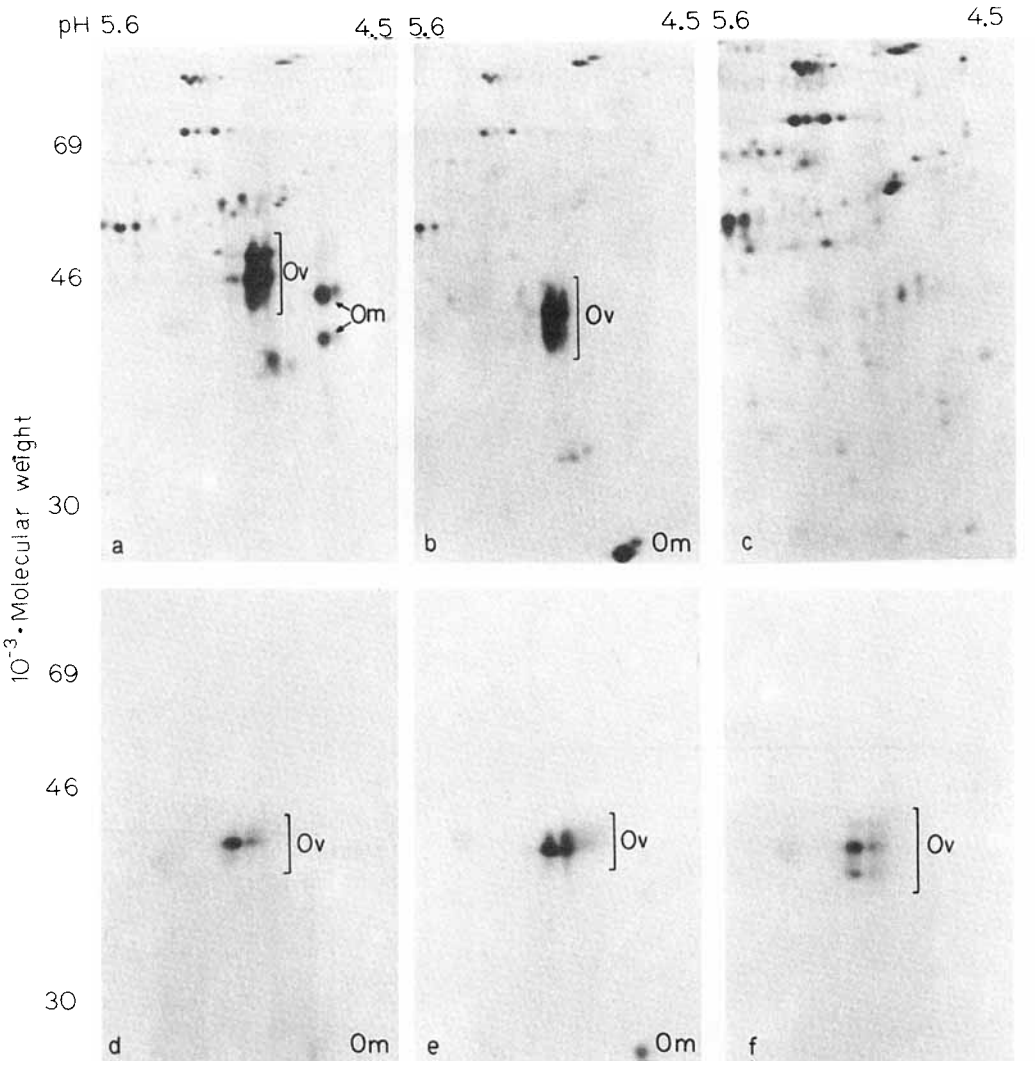

Fig. 5. *Two-dimensional electrophoresis of proteins from mRNA-injected oocytes.* Oocytes were injected with oviduct mRNA and cultured in the presence or absence of tunicamycin as described in Materials and Methods. After subcellular fractionation selected fractions were prepared for two-dimensional electrophoresis before (a, b, c) or after immunoprecipitation (d, e, f). (a) Vesicles from mRNA-injected oocytes (see Fig. 1, track 3) 300000 counts/min; (b) vesicles from mRNA + tunicamycin-injected oocytes (see Fig. 1, track 7) 300000 counts/min; (c) vesicles from uninjected oocytes (see Fig. 1, track 12) 150000 counts/min; (d) immunoprecipitated incubation media from mRNA + tunicamycin-injected oocytes (see Fig. 1, track 8) 5000 counts/min; (e) immunoprecipitate of sample run in (b) 23000 counts/min; (f) immunoprecipitate of cytosol fraction of mRNA-injected oocytes (see Fig. 3, track 6) 7200 counts/min. Ovalbumin (Ov) polypeptides are indicated by brackets, whilst ovomucoid (Om) polypeptides are indicated by arrows. The molecular weights indicated in the figure margin were ascertained from the comigration in the second dimension of the ^{14}C-labelled marker proteins (Radiochemical Centre, UK) carbonic anhydrase (30000); ovalbumin (46000); bovine serum albumin (69000)

Conclusions

Chick ovalbumin and lysozyme are secreted from oocytes of *Xenopus laevis*, thus extending the range of proteins known to be secreted from oocytes after microinjection of mRNA [1]. The relative concentrations of ovalbumin and lysozyme inside and outside the oocytes strongly indicate that the intrinsic rate of secretion of lysozyme molecules is considerably higher than that of ovalbumin molecules. We have recently confirmed this conclusion by showing that labelled lysozyme is 'chased' out of oocytes considerably faster than ovalbumin (Cutler, Lane and Colman, unpublished).

The experiments reported in this paper indicate that the rate of secretion of a variety of glycosylated proteins is unaffected by tunicamycin. These results correlate well with the lack of effect of tunicamycin on the secretion *in vivo* of ovalbumin [31,38], interferon (Table 1) and guinea-pig milk proteins (A. Boulton, unpublished). With ovalbumin and interferon these results imply that the presence of *N*-glycosylation does not facilitate secretion, whilst the unchanged electrophoretic profiles of the caseins indicate that

these molecules do not contain significant amounts of *N*-linked oligosaccharide chains. In one recent experiment we found that ovalbumin and casein secretion, though not synthesis, was considerably reduced by tunicamycin (data not shown). However, the secretion of lysozyme and α-lactalbumin, which are not glycosylated proteins, was similarly reduced and we conclude that this reduction was due to secondary effects of tunicamycin unrelated to glycosylation [39]. For many cell types an internal control of this nature is not available and secondary effects of tunicamycin cannot be formally excluded. Using the oocyte as a surrogate system to examine secretion, a control of this type can always be included by means of co-injection of the relevant mRNA preparations.

The major conclusion from the present study is that access to the lumen of the endoplasmic reticulum is a mandatory intermediate step in the secretion of ovalbumin. Once access is achieved, secretion will occur irrespective of whether glycosylation occurs. Thus it is only the intracellular location and not the status of modification of the protein that is the essential feature of ovalbumin secretion. A corollary of this finding is that if sequestered ovalbumin entered the cytosol it would not be secreted.

The unique aspect of these experiments, stemming from the use of oocytes, is that it proved possible to ensure the miscompartmentalisation of a selected secretory protein in the cytosol. The choice of ovalbumin was essential to the design and interpretation of these experiments because, unlike most other secretory proteins examined, ovalbumin lacks a detachable signal sequence [5,33]. Thus the amino acid sequence of sequestered and cytosol ovalbumin will be identical, making functional comparisons less contentious. These considerations are highlighted by the example of a rat hepatoma cell line, which synthesizes but does not secrete albumin [40]. The albumin is synthesized on membrane-free polysomes [41] and presumably does not gain access to the endoplasmic reticulum. However, these observations do not unequivocally demonstrate that passage into the endoplasmic reticulum is essential for secretion, since the trapped albumin, in retaining an uncleaved hydrophobic 'signal' peptide [42] will differ from the secreted form. Studies *in vitro* have been more revealing: it has been shown for several secretory proteins including ovalbumin [3,4,43] that they can be co-translationally, though not post-translationally, inserted into isolated microsomal vesicles. These findings argue that such insertion is a prerequisite for secretion *in vivo*. Our results are consistent with these earlier findings *in vitro* and provide formal proof that at all stages along the secretory pathway of the living cell there is a barrier to post-translational transfer. Thus the combination of microinjection and subcellular fractionation of living cells permits studies of the compartmentation of secretory proteins to be correlated with the secretion of selected (and selectable) proteins: we suggest that the oocyte, by complementing existing systems *in vitro*, will prove a generally useful system for testing hypotheses about secretion.

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Note Added in Proof. Since this article went to press, both nucleotide and amino acid sequences of human lymphoblastoid interferon have become available [Taniguichi, T., Mantei, N., Schwartzstein, M., Nagata, S., Muramatsu, M., and Weissman, C. (1980) *Nature (Lond.) 285*, 547–549; Allen, G. and Fantes, K. (1980) *Nature (Lond.) 287*, 408–411]. These show that there is little or no N-glycosylation of this interferon and this knowledge affects one aspect of the interpretation of our interferon results i.e. that glycosylation does not affect secretion. However we have now repeated the experiment shown in Table 1 with human fibroblast interferon which is glycosylated [Knight, E. (1976) *Proc. Natl Acad. Sci. USA, 73*, 520–523] and again find that interferon secretion is not affected by the presence of tunicamycin.