Meiotic Maturation in *Xenopus* Oocytes: A Link between the Cessation of Protein Secretion and the Polarized Disappearance of Golgi Apparati

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ABSTRACT We have studied the relationship between the timing of the late meiotic events that occur during progesterone-induced oocyte maturation, and intracellular protein transport. We have monitored the secretion of chick oviduct proteins from *Xenopus laevis* oocytes microinjected with polyadenylated mRNA and found that chick ovalbumin and lysozyme are not secreted during the second meiotic metaphase, in contrast to the earlier prophase stage. Maturation had no detectable effect on the glycosylation of ovalbumin, whereas it affected the glycosylation of chick ovomucoid. As maturation proceeded, the Golgi apparati disappeared in a polarized fashion, beginning in the vegetal half. This disappearance coincided temporally and spatially with that of the nuclear envelope. We speculate that Golgi apparatus disappearance and the block in secretion are causally related.

It has recently been demonstrated that intracellular protein transport is greatly decreased in cultured mammalian cells during mitosis (1). The reduction (>90%) may well be linked causally with the disappearance of the Golgi apparatus which, along with the nuclear membrane breakdown, occurs at the onset of metaphase (2–4). To study these phenomena during meiotic divisions we have used *Xenopus* oocytes. Like the oocytes of many species, the large stage VI oocyte of the amphibian *Xenopus laevis* is a cell that can remain at the diplotene stage of first meiotic prophase for several months (5). The appropriate hormonal treatment in vitro or in vivo (for review see reference 6) induces oocyte maturation to continue to second meiotic metaphase. Since the oocyte will remain physiologically viable at this stage for several hours we were interested in the fate of both secretory proteins and the many oocyte Golgi apparati during and after this meiotic maturation. As the oocyte normally secretes very few endogenous proteins we have studied the fate of chick oviduct proteins encoded on injected mRNAs; these proteins were shown previously to behave as expected in *Xenopus* oocytes (7 and 8). We demonstrate that progesterone-matured oocytes contain no Golgi apparati and can core glycosylate but not secrete chick proteins. Furthermore the disappearance of the Golgi apparati begins at the end of diplotene and occurs in a polarized fashion, in spatial and temporal correspondence with the breakdown of the oocyte nuclear membrane.

[1] *Abbreviations used in this paper:* endo H, endoglycosidase H; GVBD, germinal vesicle breakdown; polyA$^+$, polyadenylated.

MATERIALS AND METHODS

Electron Microscopy: Stage VI (9) oocytes were obtained from large females of *Xenopus laevis* as described by Colman (10). Oocytes were matured in modified Barth's saline (10) containing 0.25 μg/ml progesterone (Sigma Chemical Co., St. Louis, MO) until a white spot appeared in the animal hemisphere. The nucleus (germinal vesicle) in such oocytes had completely broken down. Control and matured oocytes were then fixed for 2 h in 1% glutaraldehyde in 0.1 M phosphate buffer, pH 6.9, containing 2 mg/ml tannic acid. Oocytes were dissected into animal and vegetal segments in fixative, rinsed in 0.1 M phosphate buffer, and postfixed using 1% OSO_4 in phosphate buffer. Oocyte fragments were dehydrated and embedded in Araldite and thin sections were cut on a Sorvall microtome (DuPont Instruments–Sorvall Biomedical Div., Wilmington, DE). Sections were stained with uranyl acetate and lead salts and examined under a Philips 301 electron microscope.

Microinjection and Culture of Oocytes: Stage VI oocytes were obtained from large *Xenopus* females and maintained in modified Barth's saline at 20°C as described previously (2 and 3). Oocytes were injected with 50 nl chicken oviduct polyadenylated (poly A$^+$)[1] mRNA (1 mg/ml) prepared as described by Cutler et al. (8). All injected oocytes were cultured for 24 h before some oocytes were matured by incubation overnight (>16 h) in saline containing 10 μg/ml progesterone. Matured and control oocytes were then injected with 50 nl [^{35}S]methionine at 10 mCi/ml (400 Ci/mmol; Amersham International, Amersham, UK) in distilled water before being cultured for 6 h more. Although in some experiments the label was dissolved in 10 mM EGTA to prevent puncture-induced activation, we noted no differences in the results. In some experiments oocytes were injected with tunicamycin (Sigma Chemical Co.) at 40 μg/ml in water, 24 h before mRNA injection.

Processing and Immunoprecipitation of Oocytes and Media: Labeled oocytes were homogenized in 40 μl/oocyte of 100 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris/HCl pH 7.6, 1% Triton X-100, 1 mM phenylmethylsulfonyl floride (Sigma Chemical Co.). After centrifugation in an Eppendorf microfuge for 30 s, the supernatants were immunoprecipitated by addition of

specific antibodies (see below) and *Staphylococcus aureus* envelopes as described previously (11). Antibodies used were a rabbit anti-chick egg white protein antibody and a rabbit anti-chicken ovomucoid antibody (a gift from Dr. M. Wickens, Dept. of Biochemistry, Wisconsin, University). The anti-egg white protein antibody cross-reacts with chicken ovalbumin, lysozyme, conalbumin, and ovomucoid. The anti-ovomucoid antibody slightly cross-reacts with ovalbumin. Unless otherwise indicated all samples were precipitated with anti-egg white antibodies.

Endoglycosidase H Digestion: Immunoprecipitates were eluted with 50 μl 100 mM Tris, pH 8.0, 1% (wt/vol) SDS, 1% (wt/vol) β-mercaptoethanol for 4 min at 100°C. After centrifugation, the supernatants were diluted with 9 vol 150 mM sodium citrate, pH 5.5. 250-μl aliquots were then incubated with or without 7.5 μl endoglycosidase H (endo H) (0.4 mU/μl; Miles Laboratories Inc., Elkhart, IN) for 18 h at 37°C. Protein was recovered by precipitation with 5 vol ice-cold acetone and subsequent centrifugation and air-drying.

Electrophoresis: Immunoprecipitates or acetone pellets were resuspended in electrophoresis sample buffer (20 mM Tris/HCl, pH 7.6, 20% glycerol, 2% SDS, 0.01% bromophenol blue) reduced, alkylated, and run on 12.5% denaturing polyacrylamide gels as described by Valle et al. (12). Gels were then fixed for 12 or 48 h and fluorographed. Longer fixation times were necessary to prevent elution of ovomucoid during fluorography. Each track received the equivalent of 0.5 oocyte homogenate or the media surrounding two oocytes.

Immunofluorescence: Large oocytes were injected with oviduct polyA$^+$ mRNA as described earlier. Some oocytes were then matured overnight in progesterone (10 μg/ml). Matured and control oocytes were then fixed for 1 h in 4% paraformaldehyde in amphibian ringer at 20°C and embedded in acrylamide following the method described by Hausen and Dreyer (13). Fixed, embedded samples were frozen by plunging into isopentane cooled to −170°C over liquid N_2. 10-μm sections were cut using a Jung Frigocut at −25°C. Sections were collected on gelatin subbed slides, air dried and fixed for 10 minutes in acetone. Slides were either used straight away or stored in airtight boxes over silica gel. Indirect immunofluorescence was carried out using anti-chick ovalbumin (Miles) and fluorescein isothiocyanate-conjugated sheep anti-rabbit IgG (Miles) following the method described in Jones and Rughani (14) or using IB4.E6, a monoclonal antibody directed against a 66K protein normally present in the cytoplasm and which is secreted into the vitelline space (Jones, E. A., unpublished data), and fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG (Miles). Fluorescence was scored using a Zeiss standard microscope fitted with epifluorescence on Tri-X-pan film.

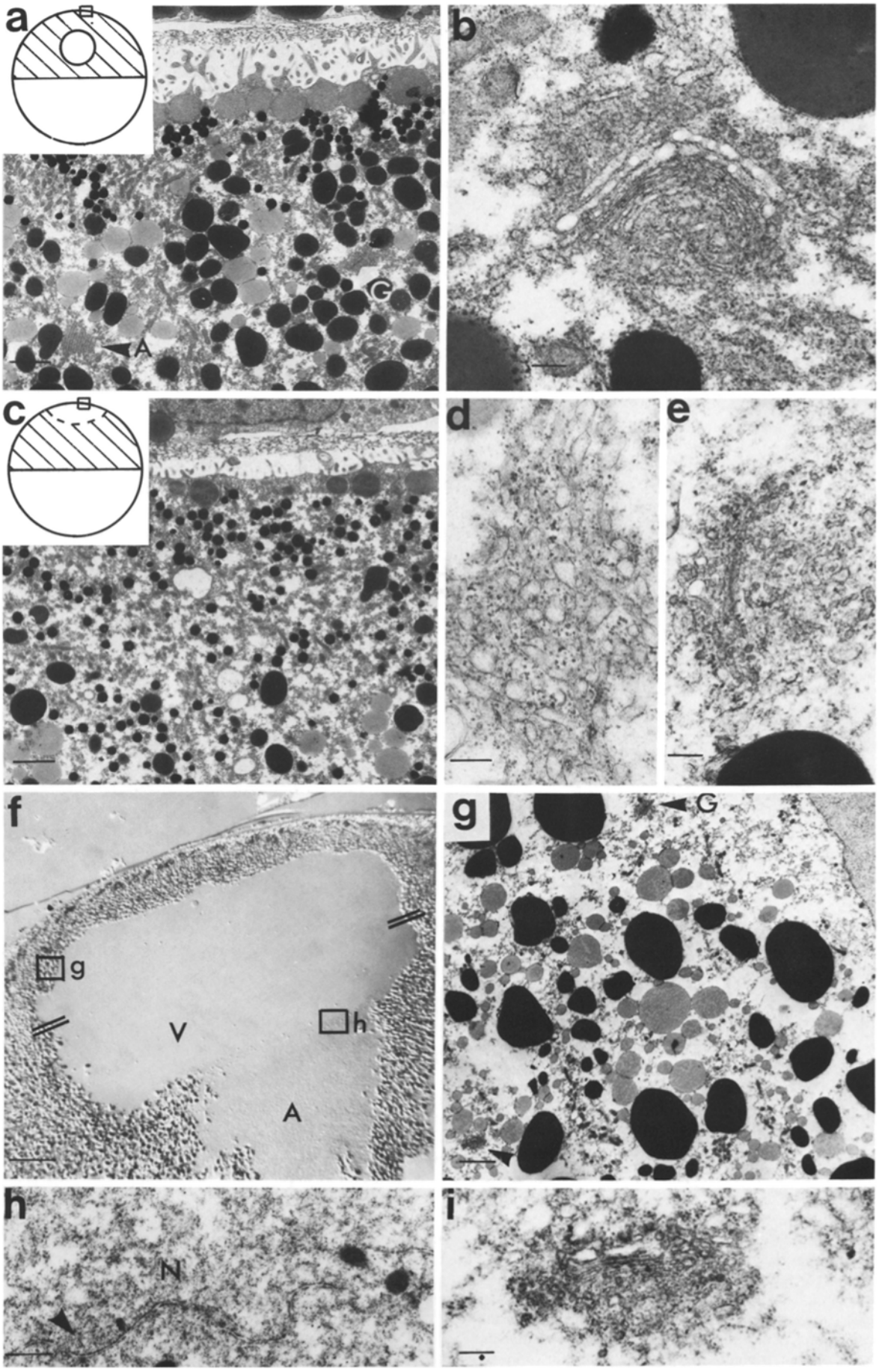
RESULTS AND DISCUSSION

One of the principal features of maturation of *Xenopus* oocytes (for reviews see references 6 and 15) is the breakdown of the oocyte nucleus (germinal vesicle). Several groups (16, 17) have demonstrated that the nuclear membrane fragments in a gradual, polarized fashion, beginning at the vegetal side of the nucleus (see Fig. 1). Many other morphological changes accompany this process of maturation including the disappearance of annulate lamellae (parallel stacks of membranes similar in structure to the nuclear membrane (18–21), retraction of surface microvilli (21), movement of cortical granules (18, 21), etc. Although an absence of Golgi apparati has been noted in mature rat oocytes (22), the fate of *Xenopus* oocyte Golgi apparati during maturation has not been described. Fig. 1 *b* shows a typical Golgi apparatus seen in a section taken near the animal pole of a control oocyte. Systematic scanning of sections taken from six control oocytes revealed Golgi apparati in animal and vegetal poles, both in cortical and deep situations (an average of five Golgi apparati per 10^{-2} mm^2 electron microscope grid space). In contrast, a similar number of oocytes that had been stimulated to mature 7 h previously and in which germinal vesicle breakdown (GVBD) was complete, had no detectable Golgi apparati (see Fig. 1 *g*). In a systematic examination of these maturing oocytes, clusters of vesicles were frequently found and the most Golgi apparatus-like of these clusters is shown in Fig. 1 *e*. In agreement with previous studies (18–21) annulate lamellae were also absent from these oocytes. An interesting situation was seen at an intermediate stage of maturation where GVBD was incomplete (Fig. 1, *f* and *i*). In latitudes where breakdown had occurred no Golgi apparati or annulate lamellae were found although many clusters of vesicles similar to those in Fig. 1 *d* could be seen. In contrast, in more cortical regions Golgi apparati and annulate lamellae were present near the intact nuclear membrane (see Fig. 1, *g* and *i*). Since the untreated oocyte is a cell frozen at the diplotene stage of first meiotic prophase whereas nuclear membrane breakdown seen in maturing oocytes, by convention, signals the beginning of metaphase, Golgi apparatus disappearance in oocytes must occur right at the beginning of metaphase.

[1] *Abbreviations used in this paper:* endo H, endoglycosidase H; GVBD, germinal vesicle breakdown; polyA$^+$, polyadenylated.

To study the effect of maturation on secretion, we injected oocytes with chick oviduct mRNA that primarily encodes ovalbumin, ovomucoid, lysozyme and conalbumin (7). Matured and control oocytes were then further injected (24 h later) with radioactive methionine in order to study the fate of newly synthesised chick proteins. Fig. 2 *a* shows that matured oocytes, in contrast to controls, make but do not secrete chick ovalbumin and lysozyme. In addition fully glycosylated chicken ovomucoid and a further chick protein, probably conalbumin (see reference 7), are not detectable in matured oocytes (Fig. 2, *a* and *b*). Nearly all the ovalbumin present in

FIGURE 1 The morphology of progesterone-matured and control oocytes. (*a*) Low-power electron micrograph of an area near the animal pole of a stage VI unmatured oocyte. *A*, annulate lamellae; *G*, Golgi apparatus. The inset illustrates the area from which this section was taken (area within the box). Shaded area represents the pigmented animal pole. Unshaded area represents the vegetal pole. Bar, 2 μm. × 3,500. (*b*) Medium-power electron micrograph of a typical Golgi apparatus in a stage VI unmatured oocyte. Bar, 0.2 μm. × 35,000. (*c*) Low-power electron micrograph of an area near the animal pole of a matured oocyte in which GVBD is complete. The inset illustrates the area from which this was taken. Bar, 2 μm. × 3,500. (*d*) Medium-power electron micrograph of a typical cluster of vesicles in a matured oocyte. Vesicles of this and other configurations are scattered throughout the cytoplasm in animal and vegetal hemispheres. Bar, 0.3 μm. × 30,100. (*e*) Medium-power electron micrograph of the only Golgi apparatus-like collection of vesicles and membranes seen in the oocyte in c. Bar, 0.2 μm. × 35,000. (*f*) Light micrograph of a section of the animal pole of a maturing stage VI oocyte in which GVBD is incomplete. Nomarski differential interference optics were used. By examining adjacent thin sections with the electron microscope the extent of GVBD was determined. All parts of the nuclear membrane on the vegetal (V) side of the double black lines were fragmented. Area *A* is a yolk-free cytoplasmic area containing mitochondria, ribosomes, and glycogen. Bar, 20 μm. × 450. (*g*) Medium-power electron micrograph of an area within the black box (*g*) in *f*. Two intact Golgi apparati (*G*) can be seen in the field of view. Bar, 2 μm. × 3,000. (*h*) Medium-power electron micrograph of an area within the black box (*h*) shown in *f*. The nuclear membrane is broken into fragments. Mitochondria and microtubules (arrowhead) can be seen between the fragments. Bar, 0.5 μm. × 19,500. (*i*) Medium-power electron micrograph of one of the Golgi apparati shown in *g*. Bar, 0.2 μm. × 35,000.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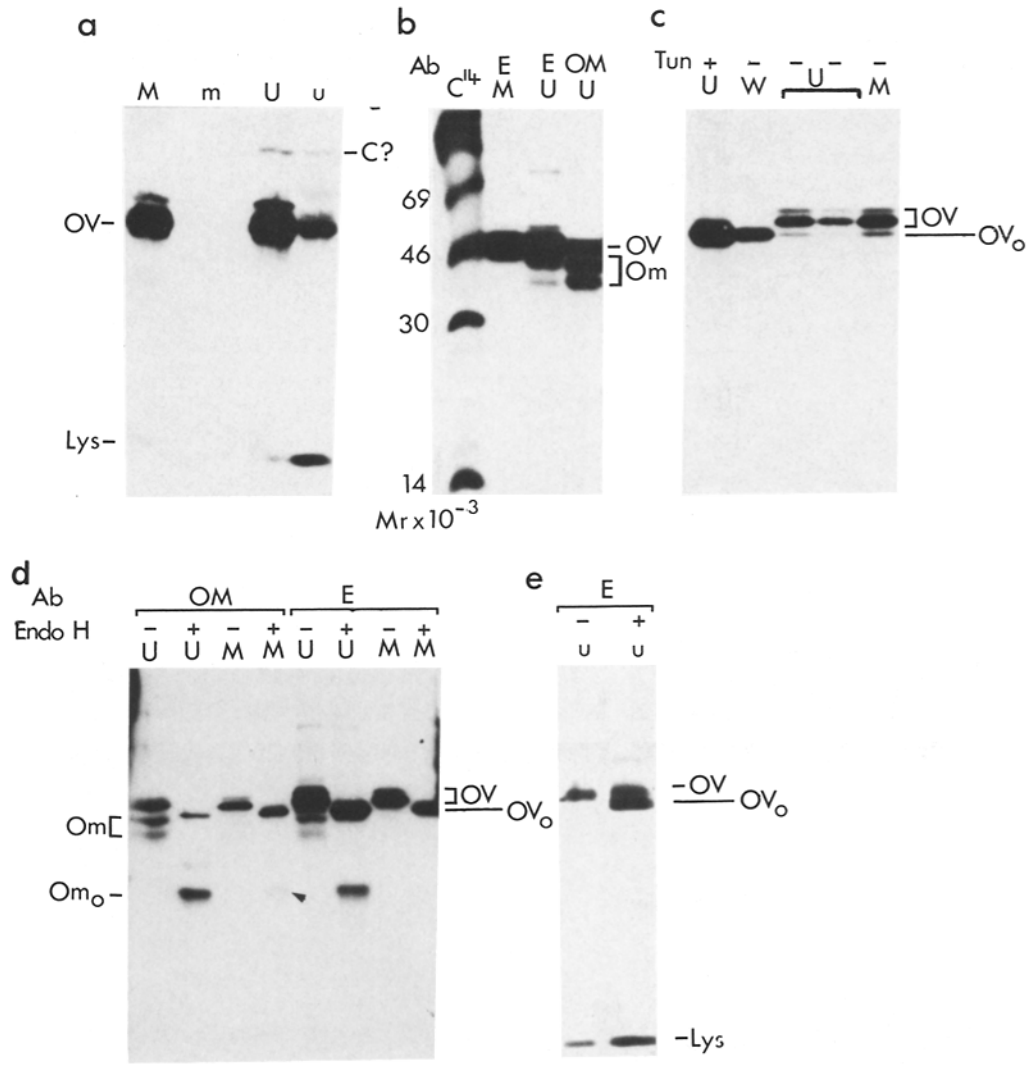
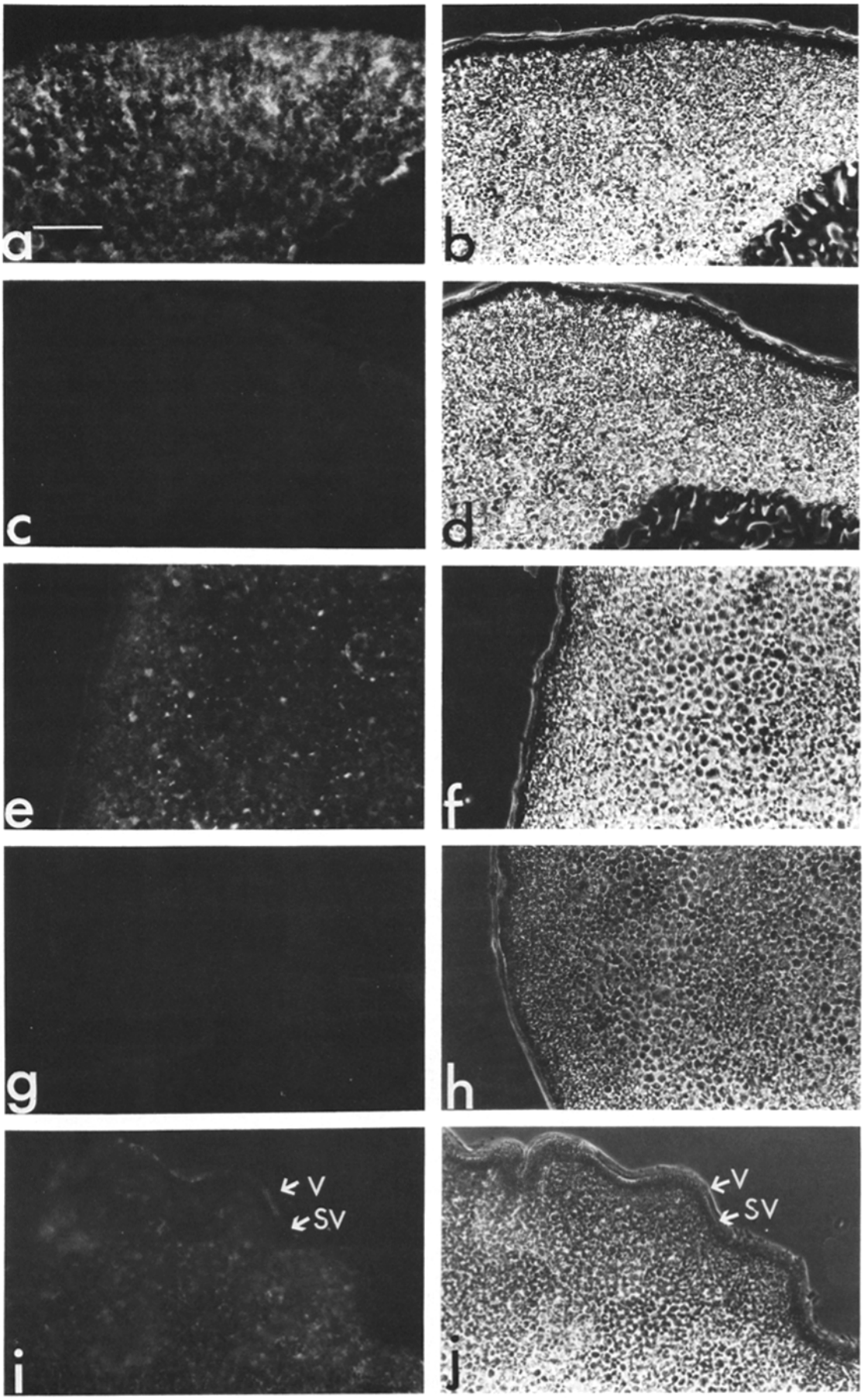


FIGURE 2 Protein secretion from *Xenopus* oocytes. Oocytes were microinjected with chick oviduct polyA$^+$ mRNA and processed as described in Materials and Methods. After culture with [^{35}S]methionine oocytes were homogenized and homogenates and incubation media were immunoprecipitated as indicated in the figure. Unless otherwise indicated all samples were immunoprecipitated with rabbit anti-chick egg white antibody. Immunoprecipitates were either run directly on 12.5% polyacrylamide gels (*a–c*) or further incubated with (+) or without (–) endo H (see Materials and Methods) before electrophoresis on 12.5% polyacrylamide gels (*d* and *e*). Each track received the equivalent of 0.5 oocyte homogenates or the media surrounding two oocytes. *a* demonstrates the cessation of ovalbumin and lysozyme secretion after oocyte maturation. *b* demonstrates the absence of fully glycosylated ovomucoid in matured oocytes. c demonstrates that *N*-glycosylation of ovalbumin is unaffected by maturation. *d* examines the endo H sensitivity of oligosaccharide side chains in the ovalbumin and ovomucoid extracted from oocytes. *e* examines the endo H sensitivity of oligosaccharide side chains in secreted ovalbumin. *U* and *u*, mRNA-injected, unmatured oocytes and surrounding media, respectively. *M* and *m*, mRNA-injected, matured oocytes and surrounding media respectively. *Ab*, antibody; *E*, anti-chick egg white antibody; *OM*, antiovomucoid. *Tun*, tunicamycin. *W*, lane contains products of wheat germ cell free translation of the oviduct polyA$^+$ mRNA. *OV* and *OV$_o$*, glycosylated and unglycosylated ovalbumins, respectively. *Om* and *Om$_o$*, glycosylated and unglycosylated ovomucoid; *Lys*, lysozyme; *C?*, putative conalbumin. C^{14}-protein markers were obtained from the Radiochemical Centre (Amersham, UK).

FIGURE 3 Immunofluorescence detection of ovalbumin in oocytes. Cryostat sections of progesterone-matured and unmatured oocytes, microinjected with oviduct polyA$^+$ mRNA, and stained with anti-ovalbumin antibody (*a*, *c*, *e*, and *g*) or IB4-E6 (see Materials and Methods). (*a*) Unmatured oocyte, injected with ovalbumin mRNA, stained with anti-ovalbumin. (*b*) Phase corresponding to *a*. (c) Uninjected unmatured oocyte stained with anti-ovalbumin. (*d*) Phase corresponding to c. (*e*) Progesterone-matured oocyte injected with ovalbumin mRNA, stained with anti-ovalbumin. (*f*) Phase corresponding to e. (*g*) Uninjected progesterone-matured oocyte, stained with anti-ovalbumin. (*h*) Phase corresponding to *g*. (*i*) Progesterone-matured oocyte labeled with monoclonal antibody IB4.E6 and fluorescein isothiocyanate–conjugated rabbit anti-mouse IgG. (*j*) Phase corresponding to *i*. *V*, vitelline envelope; *SV*, subvitelline space. Bar, 50 μm.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the matured oocytes is glycosylated as judged by the faster mobility of ovalbumin synthesized in the presence of tunicamycin (Fig. 2 *c*; see also reference 7). We have also examined the resistance of the oligosaccharide side chains of intracellular and secreted ovalbumin to digestion by endo H. It has been shown (23) that the acquisition of such resistance occurs in the Golgi apparatus, although in the case of chick ovalbumin not all molecules become resistant upon passage through the Golgi (24). With oocytes we find that all of the intracellular ovalbumin molecules of matured and control oocytes are completely sensitive to endo H (Fig. 2 *d*) whereas ~40% of the secreted protein acquires resistance (Fig. 2 *e*). We therefore conclude that in both cell types the intracellular ovalbumin is located almost exclusively in a pre-Golgi apparatus compartment, i.e., the endoplasmic reticulum. This distribution unfortunately prevents our using the endo H resistance of ovalbumin as an indicator for analysis of Golgi apparatus function in matured oocytes. Ovomucoid within control oocytes is similarly sensitive to endo H (Fig. 2 *d*); however, its absence from matured oocytes might indicate that glycosylation of this normally extensively glycosylated protein (7, 25) is disrupted in matured oocytes, a factor known to affect the stability of some (26), though not all (e.g. ovalbumin, reference 7), glycosylated proteins. In fact, the combination of incomplete glycosylation and degradation might account for the appearance only after endo H digestion of a small amount of deglycosylated ovomucoid in matured oocytes (Fig. 2 *d*, arrow).

We have shown that there is no detectable secretion from matured oocytes. However, maturation is accompanied by changes in the oocyte plasma membrane and surrounding vitelline envelope which render it less permeable to small molecules such as amino acids (27). To exclude the possibility that even in the absence of Golgi apparati, intracellular transport continued but was blocked at its last stage, exocytosis, by reduced membrane permeability, we examined mRNA-injected oocytes by indirect immunofluorescence directed at chick ovalbumin. The patterns of immunofluorescence in control and matured oocytes are different (compare Fig. 3, *a* with *e*) with a more reticular fluorescence evident in the control oocytes. The differences might reflect the general disruption of membraneous elements seen in the electron micrographs. No ovalbumin was seen to accumulate at the periphery of the oocyte as might be expected if only exocytosis was affected by maturation; observations using a monoclonal antibody against an endogenous protein present between the plasma membrane and vitelline envelope (Fig. 3 *i*) acts as a control in showing that fixation conditions allows the retention of proteinaceous material in the gap between the two membranes. Secretion ceases and intact Golgi apparati disappear during meiotic maturation in oocytes. The Golgi vanish precisely at the beginning of metaphase in synchrony with nuclear membrane breakdown. We cannot so accurately assess the meiotic stage at which secretion ceases although the two events probably are causally linked. Recently Warren et al. (28) have demonstrated that during mitosis in cultured cells, endocytosis stops after exocytosis. They speculate that Golgi apparatus integrity is maintained by a balance of vesicle fusion events (as exemplified by exocytosis) and fission events (as exemplified by endocytosis), such that an imbalance of the type they observe during mitosis with endocytosis ceasing after exocytosis would lead to Golgi apparatus fragmentation. Since endocytotic retraction of the surface microvilli of oocytes occurs during maturation (15), it will be interesting to see whether retraction occurs in a similarly vectorial fashion to the disappearance of Golgi apparati and whether it precedes or follows this event.

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