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

A. COLMAN, E. A. JONES, and J. HEASMAN*

Department of Biological Sciences, Medical Research Council Developmental Biology Group, University of Warwick, Coventry, CV4 7AL; and *Department of Anatomy, St. Georges Medical School, London, SW17 ORE, United Kingdom

**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 apparatus 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.

**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% OSO₄ 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 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⁺) 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 [³⁵S]methionine at 10 μCi/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 MgCl₂, 10 mM Tris/HCl pH 7.6, 1% Triton X-100, 1 μM phenylmethylsulfonyl fluoride (Sigma Chemical Co.). After centrifugation in an Eppendorf microfuge for 30 s, the supernatants were immunoprecipitated by addition of
appearance of annulate lamellae (parallel stacks of membranes of the nucleus (see Fig. 1). Many other morphological changes in matured 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 apparatus has been noted in mature rat oocytes (22), the fate of Xenopus oocyte Golgi apparatus during maturation has not been described. Fig. 1b 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 apparatus in animal and vegetal poles, both in cortical and deep situations (an average of five Golgi apparatus 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 apparatus (see Fig. 1g). 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. 1e. 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 l). In latitudes where breakdown had occurred no Golgi apparatus or annulate lamellae were found although many clusters of vesicles similar to those in Fig. 1d could be seen. In contrast, in more cortical regions Golgi apparatus and annulate lamellae were present near the intact nuclear membrane (see Fig. 1, g and f). 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.

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. 2a 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](https://example.com/figure1.png)
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 [35S]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; C1, putative conalbumin. C14-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 anti-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.
the matured oocytes is glycosylated as judged by the faster mobility of ovalbumin synthesized in the presence of tunicamycin (Fig. 2c; 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. 2d) whereas ~40% of the secreted protein acquires resistance (Fig. 2e). 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. 2d); 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., ovomucin, 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. 2d, 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 apparatus, 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 chch 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 membranous 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. 3i) acts as a control in showing that fixation conditions allow the retention of proteinaceous material in the gap between the two membranes. Secretion ceases and intact Golgi apparatus disappear during meiotic maturation in oocytes. The Golgi vanishes 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 apparatus and whether it precedes or follows this event.

We thank the Cancer Research Campaign and Medical Research Council for financial support.

Received for publication 14 February 1985, and in revised form 28 March 1985.

REFERENCES

1. Warren, G., C. Featherstone, G. Griffiths, and B. Burke. 1983. Newly synthesized G subunit of vesicular stomatitis virus is not transported to the cell surface during mitosis. J. Cell Biol. 97:1623–1628.

2. Zeligs, J. D., and S. H. Woolman. 1979. Mitosis in rat thymus epithelial cells in vivo. I. Ultrastructural changes in cytoplasmic organelles during the mitotic cycle. J. Ultrastruct. Res. 66:53–77.

3. Burke, R., G. Griffiths, H. Reggio, D. Louvard, and G. Warren. 1982. A monoclonal antibody against a 133-K Golgi membrane protein. EMBO (Eur. Mol. Biol. Organ.) J. 1:1621–1628.

4. Miller, G., and K. Weber. 1982. Golgi detection in mitotic and interphase cells by monoclonal to secreted galactosyltransferase. Exp. Cell Res. 142:85–94.

5. Balintsky, B. I. 1981. In An Introduction to Embryology. 5th Edition. CBS College & Professional Publishing, Philadelphia. 57–91.

6. Smith, L. D. 1975. Molecular events during oocyte maturation. In Biochemistry of Animal Development. Vol. 3. R. Weber, editor. Academic Press, Inc., New York. 1–46.

7. Colman, A., C. D. Lane, R. Craig, A. Boulton, T. Meoton, and J. Momin. 1981. The inference of topology and glycosylation on the fate of heterologous secretory proteins made in Xenopus oocytes. Eur. J. Biochem. 113:339–348.

8. Cutler, D. C., Lane, and A. Colman. 1981. Non-parallel kinetics and the role of tissue specific factors in the secretion of chicken ovalbumin and lysosome from Xenopus oocytes. J. Mol. Biol. 153:971–931.

9. Dunant, J. N. 1972. Oogenesis in Xenopus laevis (Daudin). I. Stages of oocyte development in laboratory maintained animals. J. Morphol. 136:153–179.

10. Colman, A. 1984. Translation of eukaryotic messenger RNA in Xenopus oocytes. In Transcription and Translation—a Practical Approach. B. D. Hames and S. J. Higgins, editors. IRL Press, Oxford, U.K. 271–302.

11. Valle, G., J. Belley, and A. Colman. 1981. Synthesis and secretion of mouse immunoglobulin chains from Xenopus oocytes. Nature (Lond.) 291:338–340.

12. Valle, G., J. Belley, A. Williamson, T. Meomin, and A. Colman. 1983. Post translational fate of variant MOPC 315 chains in Xenopus oocytes and mouse myeloma cells. Exp. Cell Res. 132:131–138.

13. Hausen, P., and C. Dreyer. 1981. Urea reactivates antigens in paraffin sections for immunofluorescence staining. Stain Technol. 56:287–293.

14. Jones, E. A., and A. S. Rnghani. 1984. The identification of a tissue-restricted plasma-membrane marker in Xenopus laevis embryony using a monoclonal antibody. Cell Differ. 14:78–83.

15. Ballatin, C. V., and H. J. Clarke. 1979. Oocyte maturation. Int. Rev. Cytol. 61:185–282.

16. Defellet, T. A., and M. N. Skobkina. 1969. The role of the germinal vesicle in the process of oocyte maturation in Annu and Aspertiunidae. Ann. Embryol. Morphol. Suppl. 2:133–151.

17. Brachet, J., P. Hanocq, and P. Van Gansen. 1970. A cytochemical and ultrastructural analysis of in vitro maturation in amphibian oocytes. Dev. Biol. 21:157–195.

18. Balintsky, B. I., and R. J. DeVries, 1963. Observations and differentiation of cytoplasmic structures in the oocytes of Xenopus laevis. Acta Morphol. Embryol. Exp. 6:65–108.

19. Kessell, R. G., and S. Subtelny. 1981. Alteration of Annexin lamellae in the in vivo proteoglycan treated, full grown Rana pipiens oocyte. J. Exp. Zool. 217:119–135.

20. Steinert, G. E. E., B. J. Hanocq-Querr, and J. Brachet. 1974. Ultrastructure of Xenopus laevis oocytes after injection of an extract from proteoglycan-treated oocytes. J. Ultrastruct. Res. 49:188–210.

21. Campanella, C., P. Andreuccetti, C. Taddii, and R. Tavoli. 1984. The modification of cytoplasmic endoplasmic reticulum during in vitro maturation of Xenopus laevis oocytes and its involvement in cortical granule exocytosis. J. Exp. Zool. 229:283–293.

22. Oder, L. 1960. Electron microscopic studies on ovarian oocytes and unfertilized tubal ova in the rat. J. Biophys. Biochem. Cytol. 7:567–574.

23. Robbins, P., S. Hubbard, S. Turco, and D. Wirth. 1977. Proposal for a common oligosaccharide intermediate in the synthesis of membrane glycoproteins. Cell. 12:893–900.

24. Palkin, R. 1972. Regulation of protein synthesis in chick oviduct. J. Biol. Chem. 247:6450–6461.

25. Nairn, S., H. Haruya, G. Longostr, J. P. Carver, A. A. Grey, and H. Schachter. Control of glycoprotein synthesis. 1980. J. Biol. Chem. 255:4876–4884.

26. Olden, K., R. Pratt, and M. Yamada. 1978. Role of carbohydrates in protein secretion and its regulation. Cell. 14:381–403.

27. Woodland, H. R., and E. D. Adamson. 1977. The synthesis and storage of histones during the oogenesis of Xenopus laevis. Dev. Biol. 57:118–135.

28. Warren, G., J. Davoist, and A. Crockcroft. 1984. Recycling of transferrin receptors in A51 cells is inhibited during mitosis. EMBO (Eur. Mol. Biol. Organ.) J. 3:2217–2225.