Protein Transport from Endoplasmic Reticulum to the Golgi Complex Can Occur during Meiotic Metaphase in *Xenopus* Oocytes

Aldo Ceriotti and Alan Colman

Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, United Kingdom

Abstract. We have previously shown [9] that *Xenopus* oocytes arrested at second meiotic metaphase lost their characteristic multicisternal Golgi apparati and cannot secrete proteins into the surrounding medium. In this paper, we extend these studies to ask whether intracellular transport events affecting the movement of secretory proteins from the endoplasmic reticulum to the Golgi apparatus are also similarly inhibited in such oocytes. Using the acquisition of resistance to endoglycosidase H (endo H) as an assay for movement to the Golgi, we find that within 6 h, up to 66% of the influenza virus membrane protein, hemagglutinin (HA), synthesized from injected synthetic RNA, can move to the Golgi apparati in nonmatured oocytes; indeed after longer periods some correctly folded HA can be detected at the cell surface where it distributes in a nonpolarized fashion. In matured oocytes, up to 49% of the HA becomes endo H resistant in the same 6-h period. We conclude that movement from the endoplasmic reticulum to the Golgi can occur in matured oocytes despite the dramatic fragmentation of the Golgi apparati that we observe to occur on maturation. This observation of residual protein movement during meiotic metaphase contrasts with the situation at mitotic metaphase in cultured mammalian cells where all movement ceases, but resembles that in the budding yeast *Saccharomyces cerevisiae* where transport is unaffected.

THE large stage VI oocyte (13) is a cell arrested at first meiotic prophase. On stimulation by progesterone (in vitro) or a progesterone-like substance (in vivo), meiotic division resumes and progresses to second meiotic metaphase, a process called maturation. Arrest at metaphase can persist for several hours until release is effected by fertilization or artificial activation (28). We have shown previously that in matured oocytes the secretion of chick oviduct proteins ceases and the numerous Golgi apparati are no longer visible (9). These results are consistent with the observations made on mammalian cells during mitotic metaphase where it has been shown that vesicularization and dispersal of the Golgi cisternae occurs (20, 24, 31) and transport to the plasma membrane is greatly (if not totally) inhibited (29). Recently, Featherstone et al. (14) established that the vesicular stomatitis virus G protein made in mitotic Chinese hamster ovary (CHO) cells was completely sensitive to endoglycosidase H (endo H)[1] and was poorly acylated, phenotypes that indicated that protein transfer from ER to Golgi had ceased. In our previous work (9), we were unable to assay ER-Golgi movement using endo H resistance because, within the limits of the assay procedures, all the intracellular forms of the chick glycoproteins, ovalbumin, and ovomucoid, remained endo H sensitive. In this present paper, we have examined the acquisition of endo H resistance by the viral membrane protein, influenza hemagglutinin (HA), in nonmatured and metaphase-arrested oocytes. We find that endo H-resistant HA, although reduced in amount, is still made in arrested oocytes. In contrast, no secretion of chick lysozyme and ovalbumin from the same oocytes could be detected. We interpret these observations as showing that protein transfer from ER to functional Golgi elements can occur during meiotic metaphase although one or more of the later events in protein movement is greatly inhibited.

A. Ceriotti was on leave from Instituto Biosintesi Vegetali, Consiglio Nazionale del Recerche, 20133 Milano Italy.

1. *Abbreviations used in this paper*: Endo H, endoglycosidase; HA, hemagglutinin.

Materials and Methods

Microinjection and Culture of Oocytes

Stage VI oocytes were obtained from large *Xenopus* females and maintained in modified Barths' saline at 20°C as described previously (7). Oocytes were injected with 50 nl chicken oviduct polyadenylated RNA (12) or HA synthetic RNAs (see below) at 100 μg/ml. Injected oocytes were cultured overnight with or without 5 μg/ml progesterone (Sigma Chemical Co., St. Louis, MO). Matured and nonmatured oocytes were then either processed for electronmicroscopy (see below) or cultured for indicated times in media containing [^{35}S]methionine (Amersham Corp., Arlington Heights, IL; 800 Ci/mmol) at 1 mCi/ml, 5 oocytes/30 μl. In some experiments the labeling was followed by a "chase" period where fresh media, supplemented with 1 mM methionine was used. Oocytes were frozen at −70°C before analysis.

In Vitro Transcription

The construction of the plasmids pSP64HAwt (containing full length HA cDNA prepared from A/Japan/305/57 genomal RNA) and pSP64HAenv (containing HA cDNA where the native hydrophobic signal sequence is replaced by that from the Rous sarcoma virus envelope protein) has been described previously (6). Synthetic RNA transcripts were prepared using SP6 RNA polymerase (Boehringer Mannheim Diagnostics, Inc., Houston, TX) as described previously (6).

Processing and Immunoprecipitation

Homogenized oocytes and media were immunoprecipitated using rabbit antisera raised against denatured viral HA (a gift from M.-J. Gething University of Texas at Dallas, TX) or chick egg white proteins (12) as described previously (6). Immunoprecipitates were analyzed before or after endo H (Miles Scientific, Naperville, IL) digestion (9) on 10% reducing, SDS polyacrylamide gels as described by Colman (7). Each track contained the immunoprecipitate from 2.5 homogenized oocytes or the media surrounding 5 oocytes.

Trypsin Surface Treatment

In some experiments, labeled oocytes were manually defolliculated (7) and then treated with 100 μg/ml trypsin (Sigma Chemical Co.) for 30 min at 0°C in Barths' medium. The oocytes were washed several times in fresh media and then soybean trypsin inhibitor (Sigma Chemical Co.) was added to a final concentration of 500 μg/ml. After 10 min on ice oocytes were homogenized, immunoprecipitated, and electrophoresed as described above.

Histology

Oocytes were processed for immunofluorescence as described previously (9) with the modification that paraformaldehyde-fixed oocyte sections were labeled first with anti-HA and then with fluorescein isothiocyanate-conjugated sheep anti-rabbit IgG (Miles Scientific).

Results

HA Becomes Endo H Resistant in Oocytes

Influenza HA encoded by the A/Japan/305/57 strain is a 76-kD membrane glycoprotein containing 562 amino acids (16). In influenza virus–infected mammalian cells, the protein acquires five asparagine-linked oligosaccharide side chains, all of which become resistant to endo H as a result of the removal of mannose residues by the alpha mannosidases I and II as the protein moves through the *cis* and medial Golgi compartments (18). We have subcloned the HA coding sequence into the vector pSP64T (19) and synthesized transcripts in vitro using the SP6 RNA polymerase, having previously found that transcripts made with this construct, which contains 5′ and 3′ untranslated regions of the adult *Xenopus* β globin gene, are translated extremely well in *Xenopus* oocytes (6). Oocytes were injected with HA transcripts, continuously labeled in [^{35}S]methionine (Fig. 1 *A*), or pulse labeled for 3 h and then chased in nonradioactive media for various times (Fig. 1 *B*). In several experiments (Fig. 1 *A*), though not all (Fig. 1 *B*), the apparent molecular mass of HA increases with time, indicating the conversion of simple to complex oligosaccharide side chains. Irrespective of this gel mobility change, peripheral glycosylation can be demonstrated to have occurred in every experiment by the results of endo H digestion that show the gradual acquisition of endo H resistance as a function of time. In the experiment shown in Fig. 1 *B*, the half-time by which 50% of the HA molecules become endo H resistant to some extent has been estimated, using quantitative densitometry, to be <4 h; however, we have found that the value obtained is a function of the amount of HA made, with greater synthesis leading to shorter half-times (Ceriotti, A., and A. Colman, manuscript in preparation). After 10 h, it is clear that while in some molecules all side chains are resistant, most HA molecules contain some sensitive side chains although all molecules contain at least one resistant chain. In contrast, when we examine the glycosylation status of a mutant HA protein HAenv, which in mammalian cells is confined to the ER (17), we find that virtually all of the side chains are sensitive to endo H (Fig. 4, lanes *5* and *6*). In virally infected mammalian cells, all the surface HA oligosaccharide side chains are completely resistant to endo H so that the retention of some sensitive side chains in the oocyte HA, even after a long chase, implies that HA molecules are inefficiently processed in the

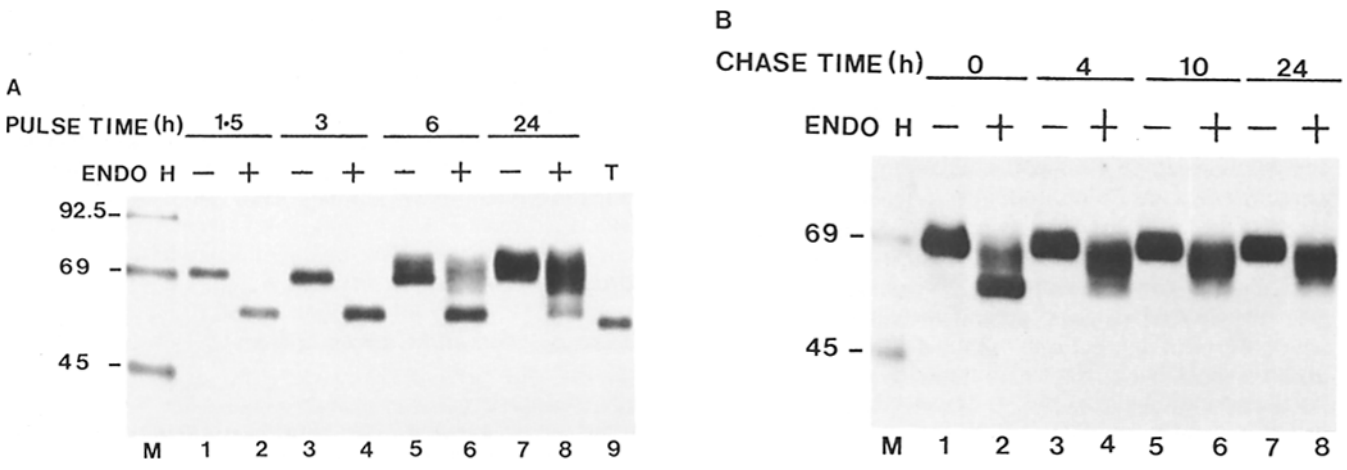

Figure 1. Time dependent acquisition of endo H resistance by HA in oocytes. Oocytes were injected with pSP64HAwt RNA (100 μg/ml), left overnight in culture medium and then radiolabeled in [^{35}S]methionine (1 mCi/ml) continuously for the times indicated (*A*) or for 3 h, followed by a chase in media containing 1 mM methionine for the times indicated (*B*). Oocytes were homogenized, immunoprecipitated, incubated with (+) or without (−) endo H, and electrophoresed on 10% SDS polyacrylamide gels. The sample T containing unglycosylated HA, was prepared from oocytes that had been coinjected with RNA and 40 μg/ml tunicamycin (Sigma Chemical Co.) 24 h before radiolabeling. *M*, ^{14}C markers, the M_r(kD) values are indicated in the margin.

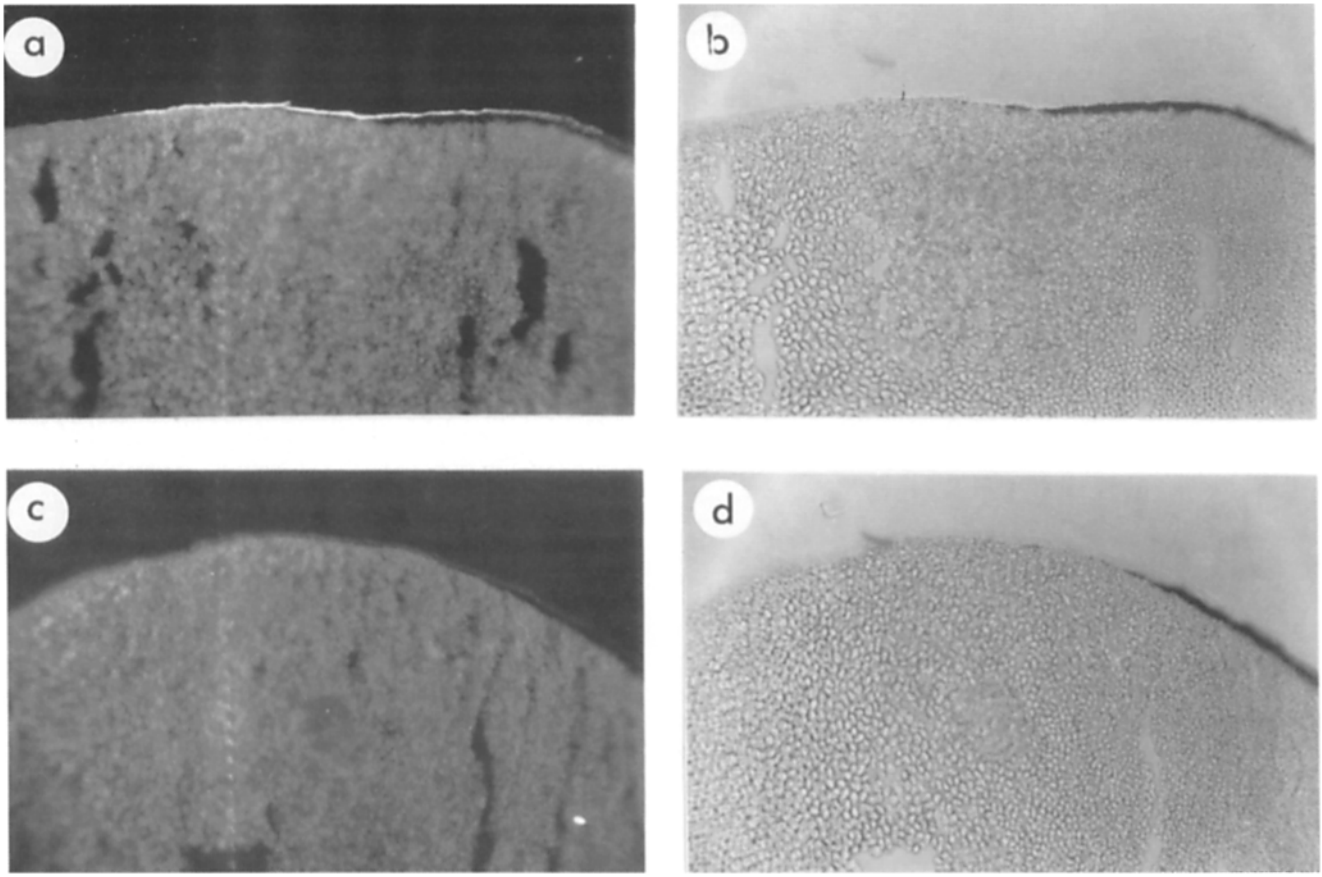

Figure 2. HA reaches the oocyte surface. Oocytes were injected with pSP64HAwt RNA (*a* and *b*) or water (*c* and *d*) and incubated for 48 h before fixation, cryosectioning into 10 μm sections, and processing with rabbit anti-HA antisera (1:100) followed by FITC-conjugated goat anti-rabbit antisera. Fluorescent (*a* and *c*) or bright field illumination (*b* and *d*) was used. The sections were taken along the animal–vegetal axis, and the pigment layer, which is concentrated in the animal cortex of the cell, is visible in *b* and *d*. The plasma membrane is clearly immunolabeled in *a* but not in *c*. The cytoplasmic signal in *a* and *c* is because of autofluorescence of yolk platelets.

oocyte Golgi. As we show below, this incomplete processing does not compromise the ability of the protein to reach the cell surface.

Movement of HA to the Plasma Membrane in Nonmatured Oocytes

Oocytes were injected with HA mRNA and incubated for 24–48 h before fixation by paraformaldehyde. After embedding and sectioning, mounted sections were labeled with rabbit anti-HA antiserum followed by fluorescently labeled goat anti-rabbit antisera. One representative section through both the nonpigmented (vegetal) and pigmented (animal) border area is shown in Fig. 2 (*a* and *b*). Although autofluorescence by intracellular yolk platelets (a known problem) obscures any specific intracellular signal, it is clear that there is specific fluorescence associated with the oocyte plasma membrane in both animal and vegetal regions. This must reflect the successful transport of some HA to the cell surface, a conclusion that was also confirmed by electronimmunomicroscopy (Griffiths, G., and A. Colman, unpublished observations) and also by the following trypsin sensitivity experiment: correctly folded HA is cleaved by trypsin at a single site into two distinctive fragments whereas HA monomers or incorrectly folded HA trimers are degraded to many small fragments (10, 17). In Fig. 3, we exposed HA-injected oocytes, which had been pulse labeled for 3 h to trypsin at 0°C before and after a 48-h chase. Under these conditions, only HA that had reached the surface should be accessible to the enzyme. Clearly some (10%, Fig. 3, see legend) of the HA present after the chase (Fig. 3, lanes *5* and *7*), but not before (Fig. 3, lanes *1* and *3*), is accessible to trypsin and is cleaved quantitatively into the two bands (HA1 and HA2) diagnostic of its previously correct conformation. We conclude that at least 10% of the HA is on the surface after 48 h. Furthermore, endo H digestion of the trypsin fragments shows that a proportion contain oligosaccharide side chains that are sensitive to the enzyme (Fig. 3, lanes *7* and *8*), supporting our contention, raised earlier, that incompletely processed HA molecules can reach the oocyte plasma membrane. It is perhaps worth commenting here that despite the polarized nature of the oocyte membrane with regard to the distribution of some membrane proteins, HA, which exclusively occupies the apical membrane of polarized, mammalian epithelial cells (for review, see reference 23), shows no regional localization in the oocyte plasma membrane.

Posttranslational Modifications of HA in Matured Oocytes

Oocytes were injected with HA and matured in progesterone

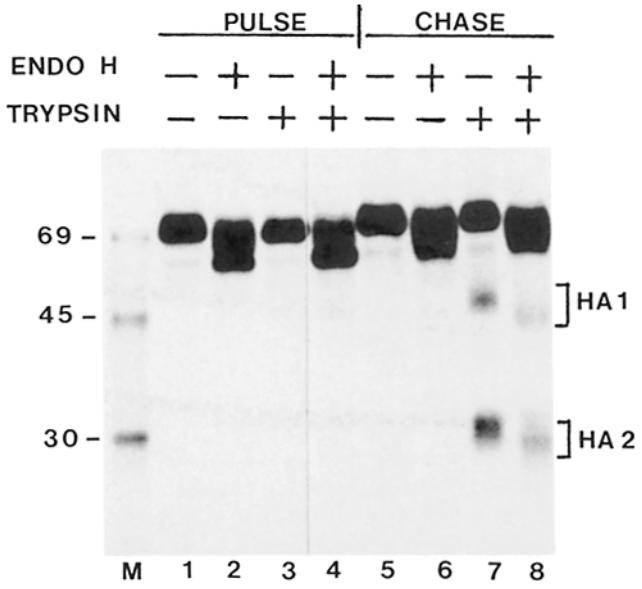

Figure 3. Surface accessibility of HA on oocytes. Oocytes were injected with SP64HAwt mRNA at 100 μg/ml, cultured overnight and pulse labeled in [^{35}S]methionine for 3 h. After washing, oocytes were either treated immediately (*PULSE*) with or without trypsin (lanes *1–4*), or first chased (*CHASE*) in unlabeled media (containing 1 mM methionine) for 48 h (lanes *5–8*). After trypsin treatment, oocytes were processed for endo H treatment as in Fig. 1. Trypsin digestion products HA1 and HA2 are indicated by brackets. Excision of the bands containing undigested and trypsin-digested HA, followed by scintillation counting showed that 10% of HA at 48 h was digested and converted to HA1 and HA2. *M*, ^{14}C markers, the M_r (kD) values are indicated in the margin.

for at least 12 h, by which time the cell cycle in the oocyte has proceeded to second meiotic metaphase. Matured and nonmatured oocytes were then labeled for 6 h in [^{35}S]methionine. Immunoprecipitated HA was then examined before and after endo H digestion. From Fig. 4, we can see that some HA made in matured oocytes acquires resistance (Fig. 4, lanes *3* and *4*) although the proportion, when compared to the nonmatured samples (Fig. 4, lanes *1* and *2*), is reduced; in these experiments, the endo H-sensitivity of the HAenv protein commented on earlier, serves as a control on the endo H digestion. Using densitometric analysis on this (Fig. 4) and two more experiments, we estimate (Table I) that under the conditions of assay, the endo H resistance ranged from 44–66% in nonmatured oocytes, and 27–49% in matured oocytes, which would seem to indicate an overall reduction in efficiency of movement of ~25–40% depending on oocyte batch.

To address the question of whether post-Golgi movement of HA could occur in matured oocytes, we had hoped to use the surface fluorescence and trypsin assays outlined above. However, matured oocytes begin to degenerate within 12–18 h of culture, and this time window proved too short to allow detection of HA arrival at the surface even in nonmatured oocytes. In view of this difficulty, we coexpressed the secretory proteins, chick ovalbumin and lysozyme, along with the HA. The secretion of the ovalbumin and lysozyme was only detected from nonmatured oocytes (Fig. 5) as we have observed before (9). Chick ovomucoid was also seen in nonmatured oocytes; its absence from matured oocytes has been previously attributed to the incomplete glycosylation and subsequent instability of this highly glycosylated protein in matured oocytes (9).

We conclude that while ER to medial Golgi movement is reduced in matured oocytes, some nonetheless occurs. However, the subsequent movement of proteins from the Golgi to the cell surface, as monitored by secretion, is inhibited.

Discussion

Protein Transport during Metaphase

During mitosis in animal cells, Golgi apparati undergo fragmentation, and the fragments disperse through the cytoplasm. This fragmentation is followed in HeLa cells by the appearance of multivesicular structures called Golgi clusters (20). Both endocytosis (3, 30) and exocytosis (29) are inhibited in mitotic mammalian cells. Furthermore, earlier transport events in the secretory pathway, such as protein movement from ER to Golgi, also appear completely inhibited (14). The simplest interpretation of these experiments is that Golgi function is inextricably linked to structure such that loss of

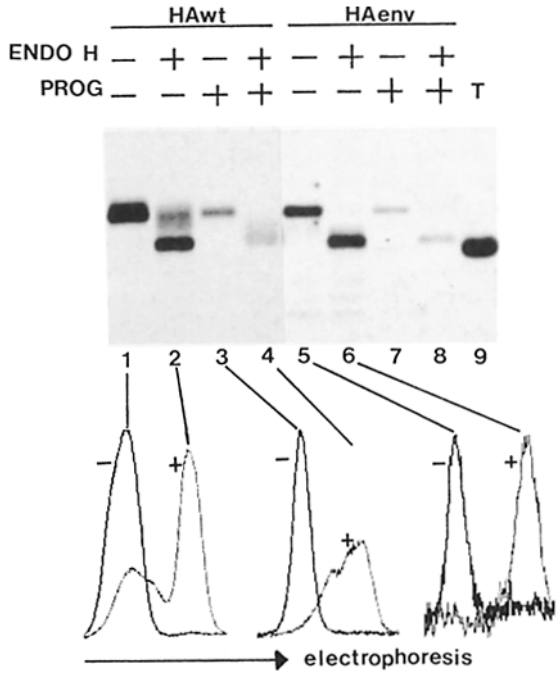

Figure 4. Terminal glycosylation of HA occurs in matured oocytes. Oocytes were injected with either pSP64HAwt (*HAwt*) or pSP64-HAenv (*HAenv*) and left overnight in culture media with or without 5 μg/ml progesterone (*PROG*). Matured oocytes and nonmatured controls were then radiolabeled for 6 h in 1 mCi/ml [^{35}S]methionine before processing as in Fig. 1. The autoradiographic exposure times of lanes 1–4 and 5–9 were 1 and 5 d, respectively. The sample T in lane 9 was prepared from oocytes that have been coinjected with HAwt mRNA and 40 μg/ml tunicamycin 24 h before labeling and contains nonglycosylated HA. The lower caption shows densitometric profiles prepared from the indicated tracks using a laser densitometer (LKB Instruments, Gaithersburg, MD). + and − refer to endo H digestion.

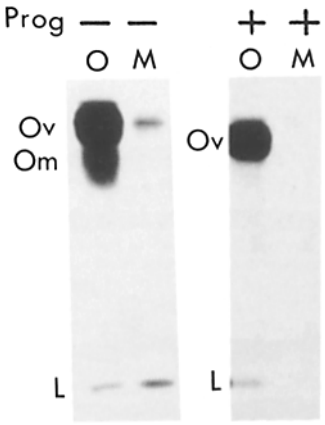

Figure 5. Secretion of ovalbumin and lysozyme does not occur from matured oocytes. In one of the experiments described in Table I, chick oviduct poly A+ RNA was coinjected with the pSP64HAwt RNA. Oocyte homogenates (*O*) and incubation media (*M*) were immunoprecipitated with anti-chick egg white antibodies and the immunoprecipitates run on a 12.5% polyacrylamide gel. *Ov*, ovalbumin; *Om*, ovomucoid; *L*, lysozyme; *PROG*, progesterone.

structure leads to loss of function. However, the functional significance of the striking architecture displayed by the stack of closely opposed Golgi cisternae remains obscure. Indeed in some situations gross disruption of Golgi morphology has been shown to have little effect on the translocation of virally encoded plasma membrane proteins (15).

Large ovarian oocytes from *Xenopus laevis* are cells arrested at first meiotic prophase. On addition of progesterone, meiosis resumes and the cell cycle progresses through to second meiotic metaphase. Further advance then requires fertilization by sperm or artificial activation. Oocytes can remain in this metaphase state for over 6 h and still be capable of normal development (Colman, A., unpublished observations). We have previously shown that metaphase-arrested oocytes lack morphologically identifiable Golgi and are unable to secrete the chick oviduct proteins, ovalbumin and lysozyme (9). In this paper, we extend these observations to show that whereas secretion of secretory proteins is blocked, movement from ER to Golgi, as monitored by the acquisition of endo H resistance by the membrane protein HA, continues, albeit at a reduced rate. We assume that resistance to endo H is conferred during passage through the Golgi and not in the ER by precedent with the mammalian situation. This assumption is consistent with our demonstration that mutant HAenv trimers, which are retained in the ER of cultured cells (17), remain endo H sensitive. However, it may be argued that in matured oocytes, ER–Golgi traffic ceases but that endo H–resistant material might nonetheless be formed because of the coaccumulation of newly synthesized HA and the mannosidases I and II. We think that this is unlikely for two reasons: (*a*) HAenv remains endo H sensitive even in matured oocytes although we might expect it to be processed by miscompartmented mannosidases unless, as a mutant protein, it is incapable of acting as a template for these enzymes; and (*b*) we observed here (Fig. 5) and previously (8, 9, 12) that secretion of ovalbumin is very slow in nonmatured oocytes (<3% secreted in 6 h), and all the intracellular ovalbumin appears to be endo H sensitive, while ~50% of the secreted material is resistant (9). From these observations and some recent intracisternal diffusion experiments (6), we have argued that the rate-limiting step in ovalbumin secretion is the ER–Golgi transport. In view of this slow movement, it is easy to understand why, if the functional mannosidases I and II are restricted to the Golgi, no intracellular endo H-resistant ovalbumin would be detected against the high background of sensitive material even in matured oocytes (9). If alternatively, active mannosidases I and II accumulate in the ER during maturation and are responsible for the 27–49% (Table I) endo H resistance seen in HA, then it is difficult to see why no comparable proportion of resistant ovalbumin is seen in matured oocytes.

Significance of Protein Transport in Metaphase-arrested Oocytes

We have shown that transport from ER to Golgi apparatus continues despite the fragmentation of the Golgi apparatus. This observation implies that, in oocytes, at least the complex architecture of the normal Golgi apparatus does not contribute greatly to the efficiency of at least some of the transport steps affecting incoming traffic from the ER. In a sense, this notion is consistent with the surprising efficiency of transport events that occur between compartments of different mammalian Golgi apparati in vivo (26) and in vitro (1). We cannot demonstrate secretion in these arrested oocytes, but, since maturation is accompanied by a decrease in plasma membrane surface area because of the retention of microvilli (5), it remains possible that secretion is reduced for this reason alone and that no other event in intracellular transport is greatly impeded during meiotic metaphase. However, such a limitation would only be effective if the secretory pathway in oocytes is saturable with the levels of foreign secretory proteins produced in these experiments. We feel this is unlikely since we have found no evidence that the secretion of oviduct proteins from oocytes is saturable (8, 12). In addition, there is no accumulation of ovalbumin beneath the plasma membrane of matured oocytes (9); such accumulation might be expected if only the final exocytotic event were inhibited.

In part this situation is reminiscent of the recent demonstration that protein transport continues uninterrupted during mitosis in the yeast *Saccharomyces cerevisiae* (22). This difference between yeast and cultured mammalian cells may be a consequence of acute selective pressure on the yeast for growth or, alternatively, it may reflect the rather atypical nature of mitosis in yeast; in this latter context it would be instructive to examine protein transport in *Schizosaccharo-*

Table I. Quantitation of Endo H Resistance in Matured and Nonmatured Oocytes

Experiment	Protein	Percentage resistant* (%)		Relative resistance(*b*/*a*)
		Nonmatured(*a*)	Matured(*b*)	
1 (as in Fig. 4)	HAwt	44	27	0.61
	HAenv	0	0	
2	HAwt	66	49	0.74
3	HAwt	63	45	0.71

In addition to the experiment described in Fig. 4 (experiment 1), two more experiments were performed in exactly the same way with two more batches of oocytes.

* Densitometric traces were obtained as in Fig. 4 and were integrated by computer to give the amount of material migrating in the positions of glycosylated and deglycosylated HA after treatment with endo H. The percentage resistant refers to the fraction (×100) of glycosylated HA in the track.

myces pombe, which exhibits a mitosis that bears closer resemblance to that occurring in higher eukaryotes. Growth considerations cannot apply to frog oocytes since negligible growth occurs during meiotic metaphase in the oocyte.

Recently it has been shown that protein movement from ER to Golgi apparatus in mammalian cells is critically dependent on the intracellular concentration of free calcium ions, with a concentration of 0.1 μM being optimal (2, 27). Since $[Ca^{2+}]_{free}$ rises transiently to 0.5–0.8 μM during mitosis in mammalian cells (25), it has been suggested that this change may contribute to the observed arrest in protein transport (2). During maturation in *Xenopus* oocytes, however, no change from the resting 0.1 μM concentration can be found (11). A transient increase to 1.2 μM does occur on fertilization, and this rise is responsible for triggering the various changes that accompany fertilization, including cortical granule exocytosis, plasma membrane depolarization, and reinitiation of the cell cycle (28). In view of the pivotal role of the increase in $[Ca^{2+}]_{free}$ at fertilization, a similar rise in $[Ca^{2+}]$ ions during maturation may have been suppressed during the evolution of female germ cells in frogs (and other species?) with the consequence that some protein transport can continue.

We wish to thank Graham Warren, John Lucocq, and John Davey for useful advice and comments on the manuscript.

We are grateful to the Cancer Research Campaign (London, UK) and the Medical Research Council (London, UK) for financial support and the European Community for a training grant to A. Ceriotti.

Received for publication 28 November 1988 and in revised form 22 May 1989.

References

1. Balch, W. E., W. G. Dunphy, W. A. Braell, and J. E. Rothman. 1984. Reconstitution of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of *N*-acetylglucosamine. *Cell.* 39:405–416.
2. Beckers, C. J., and W. E. Balch. 1988. Calcium and GTP: essential components in vesicular trafficking between the endoplasmic reticulum and golgi apparatus. *J. Cell Biol.* 108:1245–1256.
3. Berlin, R. D., and J. M. Oliver. 1980. Surface functions during mitosis II. Quantitation of pinocytosis and kinetics characterization of the mitotic cycle with a new fluorescence technique. *J. Cell Biol.* 85:660–671.
4. Berlin, R. D., J. M. Oliver, and R. J. Walter. 1978. Surface functions during mitosis I. Phagocytosis, pinocytosis and mobility of surface-bound con A. *Cell.* 15:327–341.
5. Campanella, C., P. Andreuccetti, C. Taddel, and R. Talevi. 1984. The modifications of cortical endoplasmic reticulum during in vitro maturation of *Xenopus laevis* oocytes and its involvement in cortical granule exocytosis. *J. Exp. Zool.* 229:283–293.
6. Ceriotti, A., and A. Colman. 1988. Binding to membrane proteins within the endoplasmic reticulum cannot explain the retention of the glucose regulated protein GRP 78 in *Xenopus* oocytes. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:633–638.
7. Colman, A. 1984. Translation of eucaryotic messenger RNA in *Xenopus* oocytes. *In* Transcription and Translation: A Practical Approach. B. D. Hames and S. I. Higgins, editors. IRL Press Limited, Oxford. 271–302.
8. Colman, A., D. Cutler, P. Krieg, and G. Valle. 1982. The oocyte as a secretory cell. *Ciba Found. Symp.* 98:249–267.
9. Colman, A., E. A. Jones, and J. Heasman. 1985. Meiotic maturation in *Xenopus* oocytes: a link between the cessation of protein synthesis and the polarised disappearance of Golgi apparati. *J. Cell Biol.* 101:313–318.
10. Copeland, C. S., R. W. Doms, E. M. Bolzau, R. G. Webster, and A. Helenius. 1986. Assembly of influenza hemagglutinin trimers and its role in intracellular transport. *Cell.* 53:197–209.
11. Cork, R. J., M. F. Cicerelli, and K. R. Robinson, 1987. A rise in cytosolic calcium is not necessary for maturation of *Xenopus* oocytes. *Dev. Biol.* 121:41–47.
12. Cutler, D., C. Lane, and A. Colman. 1981. Nonparallel kinetics and the role of tissue-specific factors in the secretion of chicken ovalbumin and lysozyme from *Xenopus* oocytes. *J. Mol. Biol.* 153:197–931.
13. Dumont, J. N. 1972. Oogenesis in *Xenopus laevis* (Daudin). 1. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* 136:153–179.
14. Featherstone, C., G. Griffiths, and G. Warren. 1985. Newly synthesized G protein of VSV is not transported to the Golgi complex in mitotic cells. *J. Cell Biol.* 101:2036–2046.
15. Gahmberg, N., R. F. Pettersson, and L. Kaariainen. 1986. Efficient transport of Semliki Forest virus glycoproteins through a Golgi complex morphologically altered by Uukuniemi virus glycoproteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:3111–3118.
16. Gething, M.-J., J. Bye, J. Skehel, and M. Waterfield. 1980. Cloning and DNA sequence of double-stranded copies of haemagglutinin genes from H2 and H3 strains elucidates antigenic shift and drift in human influenza virus. *Nature (Lond.).* 281:301–306.
17. Gething, M. J., K. McCammon, and J. Sambrook. 1986. Expression of wild type and mutant forms of influenza hemagglutinin: the role of folding in intracellular transport. *Cell.* 46:939–950.
18. Kornfeld, R., and S. Kornfeld. 1985. Assembly of asparagine-linked oligosaccharides. *Annu. Rev. Biochem.* 54:631–664.
19. Krieg, P. A., and D. A. Melton. 1984. Functional messenger RNAs are produced by SP6 in vitro transcription of cloned cDNAs. *Nucleic Acids Res.* 12:7057–7070.
20. Lucocq, J. M., and G. Warren. 1987. Fragmentation and partitioning of the Golgi apparatus during mitosis in HeLa cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3239–3246.
21. Lucocq, J. M., J. G. Pryde, E. G. Berger, and G. Warren. 1987. A mitotic form of the Golgi apparatus in HeLa cells. *J. Cell Biol.* 104:865–874.
22. Makarow, M. 1988. Secretion of invertase in mitotic yeast cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1475–1482.
23. Matlin, K. S. 1986. The sorting of proteins to the plasma membrane in epithelial cells. *J. Cell Biol.* 103:2565–2568.
24. Maul, G. G., and B. R. Brinkley. 1970. The Golgi apparatus during mitosis in human melanoma cells in vitro. *Cancer Res.* 30:2326–2335.
25. Poenie, M., J. Alderton. R. Steinhardt, and R. Tsien. 1987. Calcium rises abruptly and briefly throughout the cell at the onset of anaphase. *Science (Wash. DC).* 233:886–889.
26. Rothman, J. E., R. L. Miller, and L. J. Urbani. 1984. Intercompartmental transport in the Golgi complex is a dissociative process: facile transfer of membrane protein between two Golgi populations. *J. Cell Biol.* 99:260–271.
27. Schmitt, H. D., M. Puzicha, and D. Gallwitz. 1988. Study of a temperature sensitive of the *ras*-related YPT1 gene product in yeast suggests a role in the regulation of intracellular calcium. *Cell.* 53:635–647.
28. Smith, L. D. 1975. Molecular Events during Oocyte Maturation. *In* Biochemistry of Animal Development, Volume 3. R. Weber, editor. Academic Press Inc., New York. 1–46.
29. Warren, G., C. Featherstone, G. Griffiths, and B. Burke. 1983. Newly synthesized G protein of vesicular stomatitis virus is not transported to the cell surface during mitosis. *J. Cell Biol.* 97:1623–1628.
30. Warren, G. B., J. Davoust, and A. Cockcroft. 1984. Recycling of transferrin receptors in A431 cells is inhibited during mitosis. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:2217–2225.
31. Zeligs, J. D., and S. H. Wollman. 1979. Mitosis in rat thyroid epithelial cells in vivo 1. Ultrastructural changes in cytoplasmic organelles during the mitotic cycle. *J. Ultrastruct. Res.* 66:53–77.