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

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**Abstract.** We have previously shown [9] that *Xenopus* oocytes arrested at second meiotic metaphase lost their characteristic multicisternal Golgi apparatus 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 apparatus 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 apparatus 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.

**Materials and Methods**

**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 [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 electron microscopy (see below) or cultured for indicated times in media containing [35S]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.

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

The construction of the plasmids pSP64HAwt (containing full length HA cDNA prepared from A/Japan/305/57 genomic RNA) and pSP64HAenv (containing HA cDNA where the native hydrophobic signal sequence is replaced by that from the Rolls 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 Barth's 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 [35S]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](image-url)
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.

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 [35S]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, 14C markers, the Mr (kD) values are indicated in the margin.

Discussion

Protein Transport during Metaphase

During mitosis in animal cells, Golgi apparatus 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...
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 apparatus 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 | Nonmatured(a) | Matured(b) | Relative resistance(b/a) |
|------------|---------|---------------|------------|-------------------------|
| 1 (as in Fig. 4) | HAwt | 44 | 27 | 0.61 |
| 2 | HAenv | 0 | 0 | - |
| 3 | HAwt | 66 | 49 | 0.74 |
| 4 | 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.

* Denosimetric 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 [Ca²⁺]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 [Ca²⁺]free at fertilization, a similar rise in [Ca²+] 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 cannot apply to frog oocytes since negligible growth occurs during meiotic metaphase in the oocyte.

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.

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