Biogenesis of the Crystalloid Endoplasmic Reticulum in UT-1 Cells: Evidence That Newly Formed Endoplasmic Reticulum Emerges from the Nuclear Envelope

Ravindra K. Pathak, Kenneth L. Luskey, and Richard G. W. Anderson

Departments of Cell Biology and Molecular Genetics, Southwestern Graduate School of Biomedical Sciences, The University of Texas Health Science Center, Dallas, Texas 75235

Abstract. The crystalloid endoplasmic reticulum (ER), a specialized smooth ER of the compactin-resistant UT-1 cell, is composed of multiple membrane tubules packed together in a hexagonal pattern. This membrane contains large amounts of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, an integral membrane protein that enzymatically regulates endogenous cholesterol biosynthesis. Using morphological and immunocytochemical techniques, we have traced the sequence of events in the biogenesis of this ER when compactin-withdrawn UT-1 cells, which do not have a crystalloid ER, are incubated in the presence of compactin. After 15 h of incubation in the presence of compactin, many cells had profiles of ER cisternae that were juxtaposed to the nuclear envelope and studded with ribosomes on their outer membrane. Both the outer nuclear membrane and the ER membrane contained HMG CoA reductase; however, there was little or no detectable enzyme in rough ER that was free in the cytoplasm. With longer times of incubation in the presence of compactin, these cells had lamellar stacks of smooth ER next to the nuclear envelope that contained HMG CoA reductase. Coordinate with the appearance of the smooth ER, crystalloid ER appeared in the same cell. Often regions of continuity were found between the membrane of the smooth ER and the membrane of the crystalloid ER tubules. These studies suggest that HMG CoA reductase is synthesized along the outer nuclear membrane and in response to increased enzyme synthesis, a membrane emerges from the outer nuclear membrane as smooth ER cisternae, which then transforms into crystalloid ER tubules.

THE BIOGENESIS of cell membrane requires the synthesis of membrane proteins, the insertion of these proteins into an existing phospholipid bilayer, and the synthesis of additional phospholipid bilayer to house these proteins. The structural and enzymatic machineries for the synthesis and processing of certain membrane proteins have been localized to the rough endoplasmic reticulum (ER)[1] (22, 26–28) and the nuclear envelope (25). Furthermore, these same membranes may contain the enzymes that mediate phospholipid synthesis and assembly into bilayers (6). What remains to be established is how these events are coordinated during membrane biogenesis. This goal can best be achieved by studying a cell that makes large amounts of membrane in response to a specific stimulus.

The UT-1 cell offers the potential for unraveling the complex events involved in membrane biogenesis. These cells, which are derived from Chinese hamster ovary cells, are adapted to grow in high concentrations of compactin (9), a fungal metabolite that competitively inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (15), the rate limiting enzyme in cholesterol synthesis. UT-1 cells have a stably amplified gene for this enzyme (17) and when grown in the presence of 40 μM compactin express 100–500 times more HMG CoA reductase than the parental cells (9). Electron microscopic immunocytochemical studies have shown that within these cells HMG CoA reductase is embedded in numerous smooth membrane tubules that are packed together in a hexagonal pattern to form a crystalloid ER (1, 24).

An important feature of the UT-1 cell is that both the level of HMG CoA reductase and the amount of crystalloid ER are regulated by cholesterol. Cells express a maximal amount of reductase when they are grown in the presence of compactin, which inhibits the activity of HMG CoA reductase and thereby creates a state of cholesterol deprivation. When compactin is removed from the culture media, the cells can synthesize cholesterol and this cholesterol inhibits the transcription of the HMG CoA reductase gene (10). The buildup of cholesterol also stimulates the degradation of reductase (12) and causes the crystalloid ER to disappear from the cell (24). Cells grown in the absence of compactin do not have a crystalloid ER; however, when compactin is added back to

1. *Abbreviations used in this paper:* ER, endoplasmic reticulum; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

the culture medium the deprivation of cholesterol induces the synthesis of HMG CoA reductase and the crystalloid ER reappears. This induction affords the opportunity to examine in detail the sequence of events involved in the biogenesis of the crystalloid ER membrane.

Materials and Methods

Materials

Ham's F-12 medium (cat. no. 320-1765) and Dulbecco's phosphate-buffered saline (PBS, cat. no. 310-4190) were obtained from Gibco (Grand Island, NY); affinity-purified goat anti-rabbit IgG conjugated to tetramethyl rhodamine isothiocyanate from Zymed (Burlingame, CA); affinity purified goat anti-rabbit IgG conjugated to horseradish peroxidase from Biosys, Inc. (Campiegne, France); all fixatives from Electron Microscopy Sciences (Fort Washington, PA); araldite from Ladd Research Industries, Inc. (Burlington, VT); Lowicryl K4M from Polysciences, Inc. (Warrington, PA); tetrachloroauric acid (cat. no. 50780) from Fluka Chemical Corp. (Hauppauge, NY); S. aureus protein A from Pharmacia Fine Chemicals (Piscataway, NJ); and cycloheximide from Sigma Chemical Co. (St. Louis, MO). Newborn calf lipoprotein-deficient serum ($d > 1.215$ g/ml) was prepared by ultracentrifugation (9). Rabbit anti-reductase IgG is directed against HMG CoA reductase purified from the livers of colestipol-fed rats and was shown to be monospecific in previous studies (9). Control rabbit IgG was prepared from nonimmunized animals. The IgG from both immune and nonimmune rabbit antisera were purified by protein A–Sepharose chromatography (3). Compactin was kindly provided by A. Endo (Tokyo Noko University).

Cell Culture

The UT-1 cell is a cell line derived from CHO-K1 cells that was adapted to compactin resistance as previously described (9) and grown continuously in 40 μM compactin. UT-1-cw cells are UT-1 cells that were maintained in the absence of compactin for 4–7 mo. Cells were grown in a monolayer in a 5% CO_2 incubator at 37°C in medium A (Ham's F-12 medium containing 8% [vol/vol] lipoprotein-deficient serum, 2% [vol/vol] fetal calf serum, 100 U/ml of penicillin, 100 μg/ml of streptomycin, 2 mM glutamine, and 25 mM Hepes [pH 7.4]). Stock cultures were maintained in the presence (UT-1 cells) or absence (UT-1-cw cells) of 40 μM compactin.

To induce the synthesis of HMG CoA reductase and the crystalloid ER, UT-1-cw cells (7.5×10^4 cells) were seeded in 60-mm petri dishes, grown in media A for 5 d, and incubated in the presence of 40 μM compactin for the indicated time before harvest. The additions of compactin (from a 10 mM stock solution) were staggered so that all dishes were harvested on day 5. For each experiment, one set of dishes was processed for morphological and immunocytochemical studies and one set was used to measure HMG CoA reductase activity. To inhibit protein synthesis, 200 μM cycloheximide was added for 4 h before harvesting the cells.

Embedding Procedures

For indirect protein A–gold labeling, cells were fixed with 4% paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.2 for 1 h at room temperature and embedded in Lowicryl K4M as previously described (1, 24). A companion set of cells was fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4, postfixed with 2% OsO_4 in 0.1 M sodium cacodylate buffer, and embedded in araldite.

Indirect Protein A–Gold Labeling

15-nm colloidal gold particles were prepared by reducing 0.01% aqueous solution of tetrachloroauric acid with 0.1% trisodium citrate (14). The conjugation of protein A to 15-nm gold and the localization of HMG CoA reductase on thin sections of Lowicryl K4M–embedded cells was done as previously described (24). Labeled grids were stained with 4% (wt/vol) uranyl acetate (15 min) and 1% (wt/vol) lead citrate (5 min).

Quantification of Nuclear Membrane Labeling

Quantification was done directly with the electron microscope. On day 5 of cell culture, UT-1-cw cells that had been incubated with 40 μM compactin for various times were processed for protein A–gold labeling of anti–HMG CoA reductase IgG binding sites. Two grids from each time point were examined. Cells showing good morphology were selected at random and scored for the presence or absence of gold labeling around the nuclear envelope. Positive cells were divided into those that had less than one half the circumference of the nucleus labeled, and those that had greater than one half the circumference of the nucleus labeled. 50–200 cells were counted for each time point.

Indirect Immunoperoxidase Labeling of HMG CoA Reductase

HMG CoA reductase was localized by indirect immunoperoxidase electron microscopy as previously described (2). Briefly, cells were fixed with 2% paraformaldehyde in 37.5 mM sodium phosphate buffer containing 10 mM sodium periodate and 75 mM lysine at pH 6.2 for 1 h at room temperature. Cells were permeabilized with buffer A (Ca^{++}- and Mg^{++}-free PBS containing 0.1% [wt/vol] ovalbumin and 0.1% [vol/vol] saponin) and incubated with either a non-immune or an anti–HMG CoA reductase IgG (0.5 mg/ml) in buffer A for 1 h at 37°C. After washing, the cells were processed for indirect immunoperoxidase localization using affinity purified goat anti-rabbit IgG conjugated to horseradish peroxidase at a concentration of 50 μg/ml in buffer A. The cells were then incubated with 0.2% (wt/vol) diaminobenzidine and 0.01% (vol/vol) hydrogen peroxide to visualize horseradish peroxidase, postfixed in 2% OsO_4 and 1% (wt/vol) potassium ferrocyanide in 0.1 M sodium cacodylate (pH 7.3), and embedded in araldite.

Indirect Immunofluorescence

Cells grown on glass coverslips for the indicated times were fixed with 3% paraformaldehyde in 10 mM Na phosphate buffer (pH 7.4) containing 2 mM $MgCl_2$ and 150 mM NaCl for 20 min at room temperature. After washing with 50 mM NH_4Cl, cells were permeabilized with 0.1% (vol/vol) Triton X-100 and incubated with anti–HMG CoA reductase IgG (0.5 mg/ml) for 1 h at 37°C. Cells were then washed and incubated with 50 μg/ml anti-rabbit IgG conjugated to tetramethyl rhodamine isothiocyanate for 1 h at 37°C. Photographs were taken with a Zeiss photomicroscope III using an automatic photometer system.

Other Assays

The activity of HMG CoA reductase was measured in detergent-solubilized cell extracts as described (8). Protein was measured by the method of Lowry et al. (16). All electron micrographs were taken with a JEOL 100 CX electron microscope.

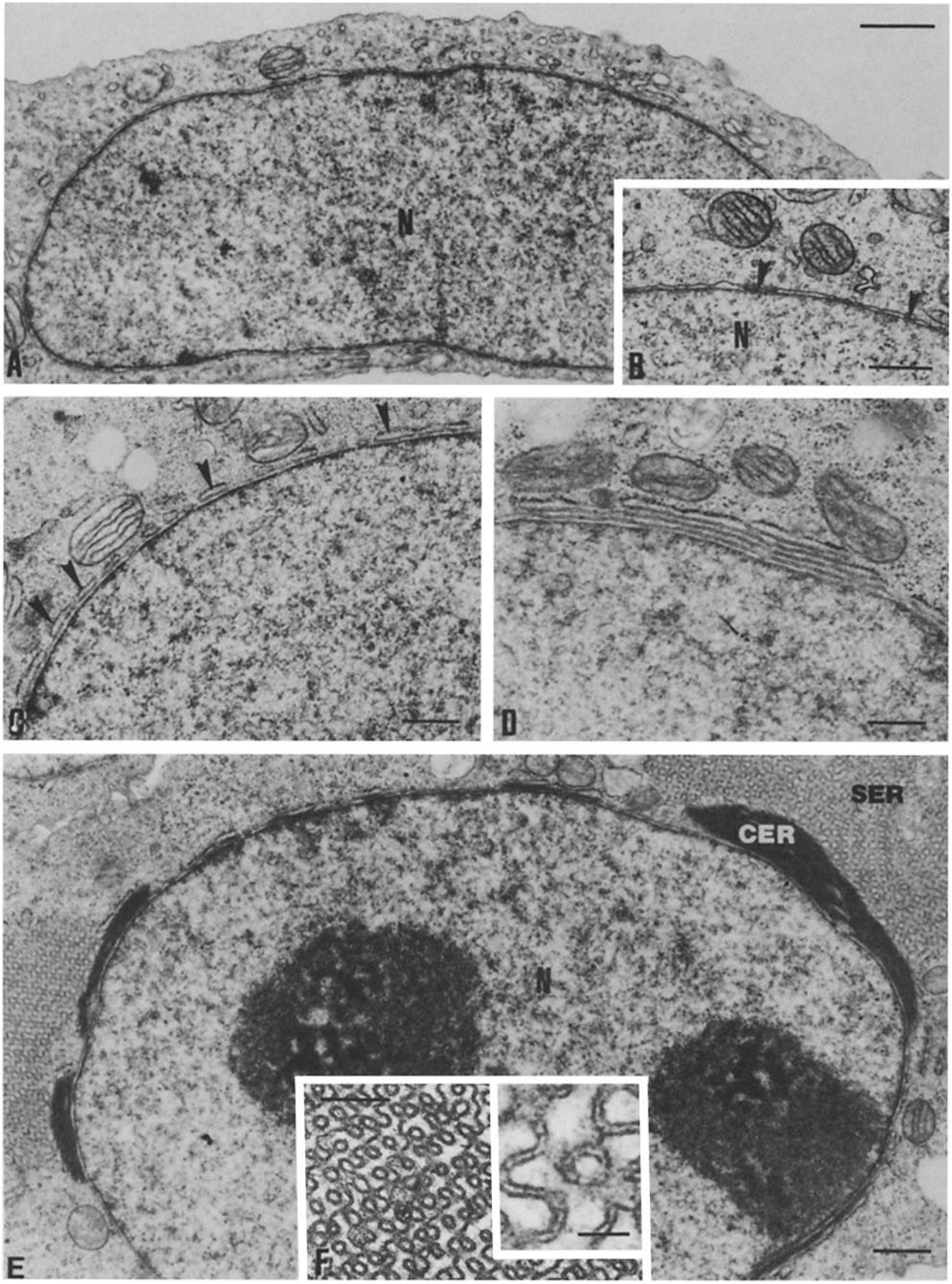
Results

In UT-1 cells, the crystalloid ER occupies 15% of the volume density of the cell (1). The major goal of the present study was to determine the origin of this morphologically distinctive membrane system. We reasoned that this could be done in three stages: (*a*) identify new membrane structures that form during the biogenesis of the crystalloid ER; (*b*) demonstrate that this membrane contains HMG CoA reductase; and (*c*) show that this HMG CoA reductase is newly synthesized enzyme. This was accomplished by using a combination of electron microscopy and immunocytochemistry to map the emergence of membrane that contains HMG CoA reductase when compactin withdrawn UT-1 cells (designated UT-1-cw cells) were incubated in the presence of compactin for various times.

Emergence of Smooth ER from the Nuclear Membrane

The sequence of events that take place during the biogenesis of the crystalloid ER was determined by incubating UT-1-cw cells in the presence of 40 μM compactin for 0, 15, 24, 48, and 72 h and processing these samples for electron microscopy. The results of this analysis are presented in Fig. 1.

UT-1-cw cells grown in the absence of compactin had the morphological characteristics of the parental Chinese hamster ovary cell (Fig. 1*A*). Within the cytoplasm were numerous profiles of rough ER and Golgi apparatus. The nuclear enve-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lope consisted of a normal double membrane that was interrupted at regular intervals by nuclear pores (Fig. 1 *B*). There were few ribosomes on the outer nuclear membrane; moreover, there was not any rough ER or smooth ER associated with the nuclear envelope.

UT-1-cw cells had a strikingly different appearance after incubation in the presence of compactin for 24–72 h. At early times of compactin treatment (15–24 h), in most cells there were many short segments of ER tightly apposed to the nuclear envelope (Fig. 1 *C*). Whereas the portion of this ER membrane next to the nuclear envelope was smooth, the portion of membrane facing the cytoplasm was studded with ribosomes. At later times of compactin treatment (24–48 h), we found stacks of smooth ER profiles next to the nuclear envelope (Fig. 1 *D*); however, the outer membrane of the outermost stack was decorated with ribosomes. An extreme example of these smooth ER stacks is shown in Fig. 1 *E*. Many sets of smooth ER were compressed together to form a myelin-like configuration of membrane next to the nuclear envelope.

The images in Fig. 1 suggest that the first event in the biogenesis of the crystalloid ER is the synthesis of large amounts of smooth ER that emerge from the nuclear envelope. Fig. 2 shows a diagram that interprets this process. HMG CoA reductase is initially synthesized by ribosomes that are bound to the outer nuclear membrane (Fig. 2 *A*). As reductase is inserted, new phospholipid bilayer is assembled, causing the formation of outpockets from the outer nuclear membrane (Fig. 2 *B*). Coordinate with the synthesis of HMG CoA reductase and the assembly of the phospholipid bilayer these outpockets expand to form smooth ER cisternae (Fig. 2 *C*). The ribosomes that remain positioned on the outer membrane of these ER cisternae synthesize more HMG CoA reductase, which results in further expansion of the membrane and the formation of additional smooth ER cisternae (Fig. 2 *D*). This process gives rise to stacks of smooth ER membrane that can be used for the assembly of the crystalloid ER tubules.

Smooth ER Transforms into Tubules of the Crystalloid ER

Within the same cells that contained stacks of smooth ER associated with the nuclear envelope, we usually found two other distinct morphological forms of ER membrane. One form was the characteristic tubules of the crystalloid ER. The other form (Fig. 1 *F*) consisted of two tightly apposed membrane bilayers that were arranged in a continuous, sinusoidal pattern (Fig. 1 *F*, inset). Usually, multiple lengths of sinusoidal membrane were stacked together in association with either the smooth ER cisternae (Fig. 1 *E*) or the crystalloid ER (Fig. 3).

The sinusoidal ER appeared to be an intermediate in the transformation of smooth ER membrane into tubular membrane of the crystalloid ER. Fig. 3 shows a cell that has regions of continuity between the membranes of the smooth ER and the sinusoidal ER (open arrows, Fig. 3) and between the membranes of the sinusoidal ER and the tubules of the crystalloid ER (solid arrows, Fig. 3). The positioning of these three forms of membrane relative to the nuclear envelope emphasizes the precursor–product relationship between the smooth ER and the crystalloid ER. Occasionally we saw smooth ER membrane in direct continuity with crystalloid ER tubular membrane.

Membrane Emerging from the Outer Nuclear Membrane Contains HMG CoA Reductase

Fig. 2 predicts that smooth ER membrane appears in response to the synthesis of HMG CoA reductase on the outer nuclear membrane. Therefore, we next applied indirect immunocytochemical techniques to map the distribution of reductase in the various membranes that arise in UT-1-cw cells incubated in the presence of compactin.

Fig. 4 shows the results of an experiment to determine by indirect immunofluorescence the distribution of HMG CoA reductase in compactin-treated UT-1-cw cells. In untreated UT-1-cw cells (Fig. 4 *A*), there was not any detectable anti-HMG CoA reductase IgG binding. However, after a 48-h incubation in the presence of compactin (Fig. 4 *B*), numerous brightly fluorescent spots were found. Many of these fluorescent spots were associated with the nucleus of the cell. Moreover, the nucleus of most cells at this time were rimmed by fluorescence, indicating that the antibody bound to the nuclear envelope. On the other hand, UT-1 cells grown continually in the presence of compactin (Fig. 4 *C*) contained numerous large fluorescent bodies that correspond to crystalloid ER (9). Only a few of these cells had labeling of the nuclear envelope.

To obtain high resolution information about the distribution of HMG CoA reductase in compactin-treated UT-1-cw cells, we used indirect protein A–gold labeling (Fig. 5). When thin sections of UT-1-cw cells embedded in Lowicryl K4M were treated with anti-HMG CoA reductase IgG followed by protein A–gold, we did not detect any gold labeling of the nuclear envelope, rough ER, or other intracellular membranes (Fig. 5 *A*). By contrast, after UT-1-cw cells were incubated in the presence of compactin for 20–48 h, the membranes asso-

Figure 1. Electron micrographs of representative stages in the biogenesis of the crystalloid ER. UT-1-cw cells grown in culture for 5 d were incubated in the presence of 40 μM compactin for 0 h (*A* and *B*), 24 h (*C*), 48 h (*D*), and 72 h (*E* and *F*) before fixation and processing as described in Materials and Methods. (*A*) The perinuclear area of a UT-1-cw cell before incubation in the presence of compactin. *N*, nucleus. Bar, 1 μm. (*B*) High magnification view of the nuclear envelope in UT-1-cw cells before incubation in the presence of compactin. *Arrows*, nuclear pores; *N*, nucleus. Bar, 0.5 μm. (*C*) The perinuclear area of a UT-1-cw cell that had been incubated in the presence of compactin for 24 h. Individual profiles of smooth ER (*arrows*), which are studded with ribosomes on the outer membrane, lie in apposition to the nuclear envelope. Bar, 0.5 μm. (*D*) The perinuclear area of a UT-1-cw cell that had been incubated in the presence of compactin for 48 h. Multiple profiles of smooth ER are stacked next to the nuclear envelope. Bar, 0.3 μm. (*E*) Low magnification view of the perinuclear area of a UT-1-cw that had been incubated in the presence of compactin for 72 h. Multiple smooth ER cisternae (*CER*) are compressed against the nuclear envelope. Adjacent to this membrane are stacks of sinusoidal ER (*SER*) membrane. *N*, nucleus. Bar, 0.6 μm. (*F*) High magnification views of the sinusoidal smooth ER. Bar, 0.5 μm. (*Inset*) Bar, 0.1 μm.

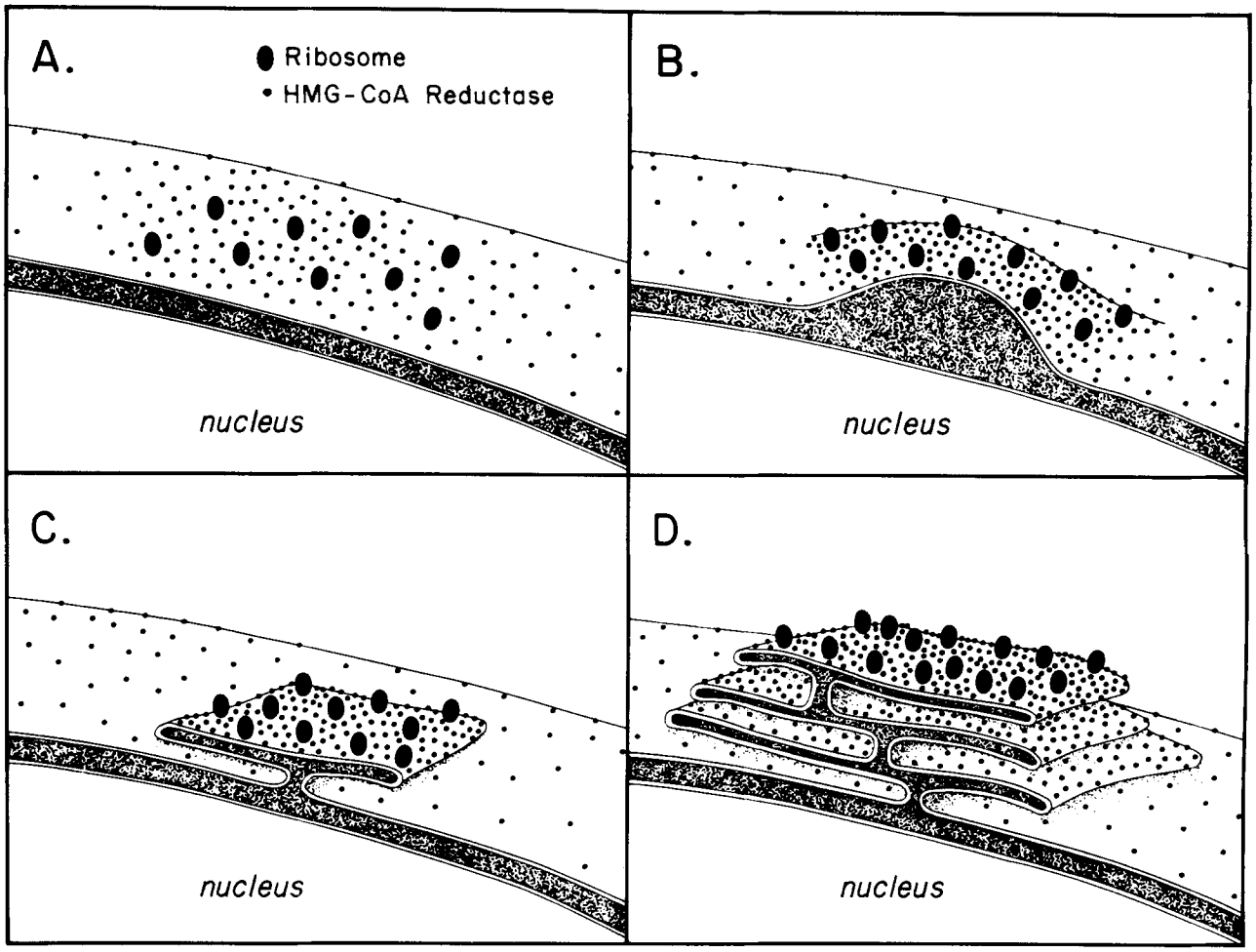


Figure 2. Illustration of how smooth ER emerges from the outer nuclear envelope in UT-1-cw cells incubated in the presence of compactin. (*A*) Ribosomes that contain nascent HMG CoA reductase dock on the outer nuclear membrane. (*B*) As the outer nuclear membrane acquires HMG CoA reductase, the membrane buds out to accommodate the expansion of the phospholipid bilayer. (*C*) Eventually these outpockets of the nuclear membrane expand laterally to form smooth ER cisternae. These cisternae remain connected to the nuclear envelope by a tenuous membrane bridge. (*D*) Additional smooth ER cisternae form from the membrane of the adjacent cisternae by a similar mechanism.

ciated with the nuclear envelope were distinctly labeled (Fig. 5, *B* and *C*). The heaviest labeling was in regions around the nucleus where there were several smooth ER profiles tightly apposed to the nuclear membrane. These ER profiles, which were capped by ribosome-studded membrane, appeared to be in continuity with forming crystalloid ER (arrows, Fig. 5*C*) that was also decorated with gold. When stacks of smooth ER were found (Fig. 5*D*), they were also heavily labeled by the anti-HMG CoA reductase IgG. Fig. 5*E* shows these relationships within a single cell. Next to the nuclear envelope were multiple stacks of smooth ER that were sandwiched between the nucleus and the longitudinal tubular profiles of the crystalloid ER. Both types of membrane were heavily labeled.

Table I shows that the amount of nuclear envelope-associated protein A-gold labeling increased with time after UT-1-cw cells were incubated in the presence of compactin. After 0 h and 8 h of incubation in the presence of compactin, there was little change in the level of HMG CoA reductase and few cells were found that had any protein A-gold labeling on the periphery of the nucleus. By 22 h of compactin treatment, however, there was a fivefold increase in HMG CoA reductase activity and ~45% of the cells had protein A-gold labeling around the nuclear envelope. With further incubation in the presence of compactin, there was a progressive increase in both the number of cells that contained nuclear envelope-associated labeling and the extent of the nuclear envelope that was labeled. By 72 h of incubation in the presence of compactin, 92% of all cells had labeled nuclear envelope and in 57% of the cells one-half or more of the circumference of the nucleus was labeled.

As an adjunct to the protein A-gold labeling techniques, we used indirect immunoperoxidase to detect anti-HMG CoA reductase IgG binding sites in differentiating UT-1-cw cells. Our purpose was twofold: (*a*) to determine if rough ER that was scattered throughout the cytoplasm of the cell contained HMG CoA reductase; and (*b*) to determine whether or not the nuclear membrane, in addition to the smooth ER membranes next to the nuclear envelope, contained HMG CoA reductase. The high sensitivity and resolution of this type of indirect immunostaining allowed us to make these determinations.

Fig. 6 shows a low power electron micrograph of a UT-1-cw cell that had been incubated in the presence of compactin for 48 h and processed for indirect immunoperoxidase local-

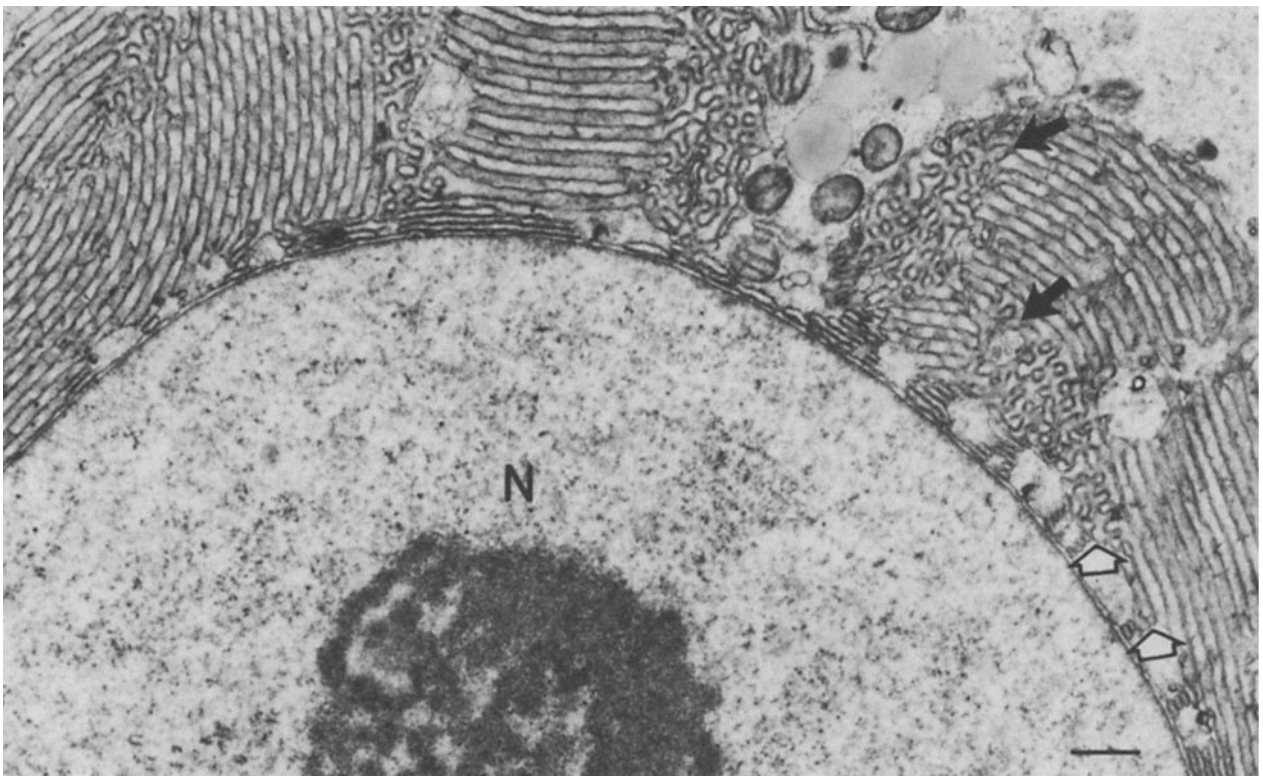


Figure 3. Electron micrograph showing smooth ER cisternae, sinusoidal ER, and crystalloid ER in the same cell. UT-1-cw cells were incubated in the presence of compactin for 72 h. Smooth ER membrane apposed to the nuclear envelope are in continuity with the sinusoidal ER membrane (*open arrows*). The latter membrane is in continuity with the tubules of the crystalloid ER (*solid arrows*). *N*, nucleus. Bar, 0.5 μm.

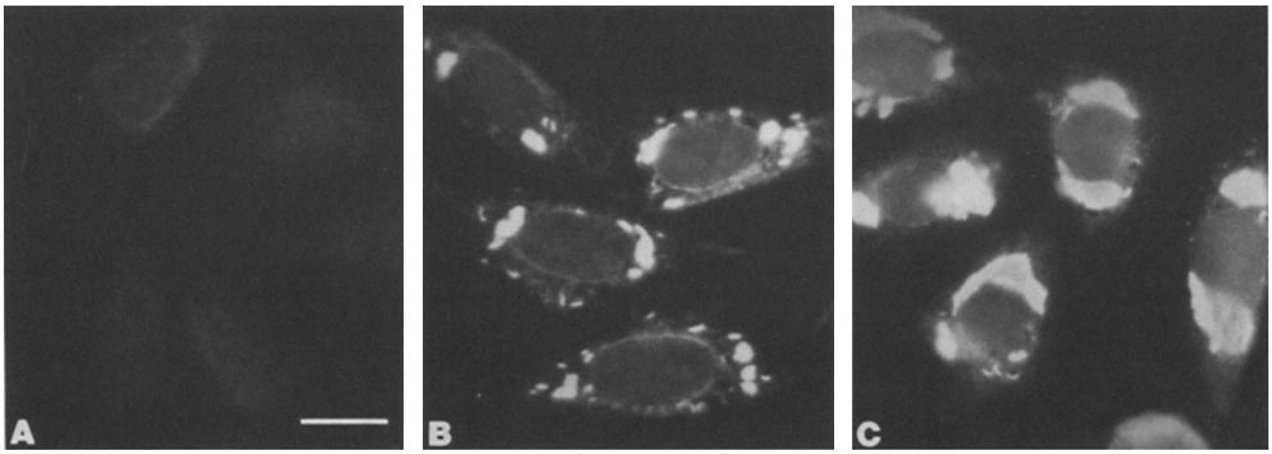
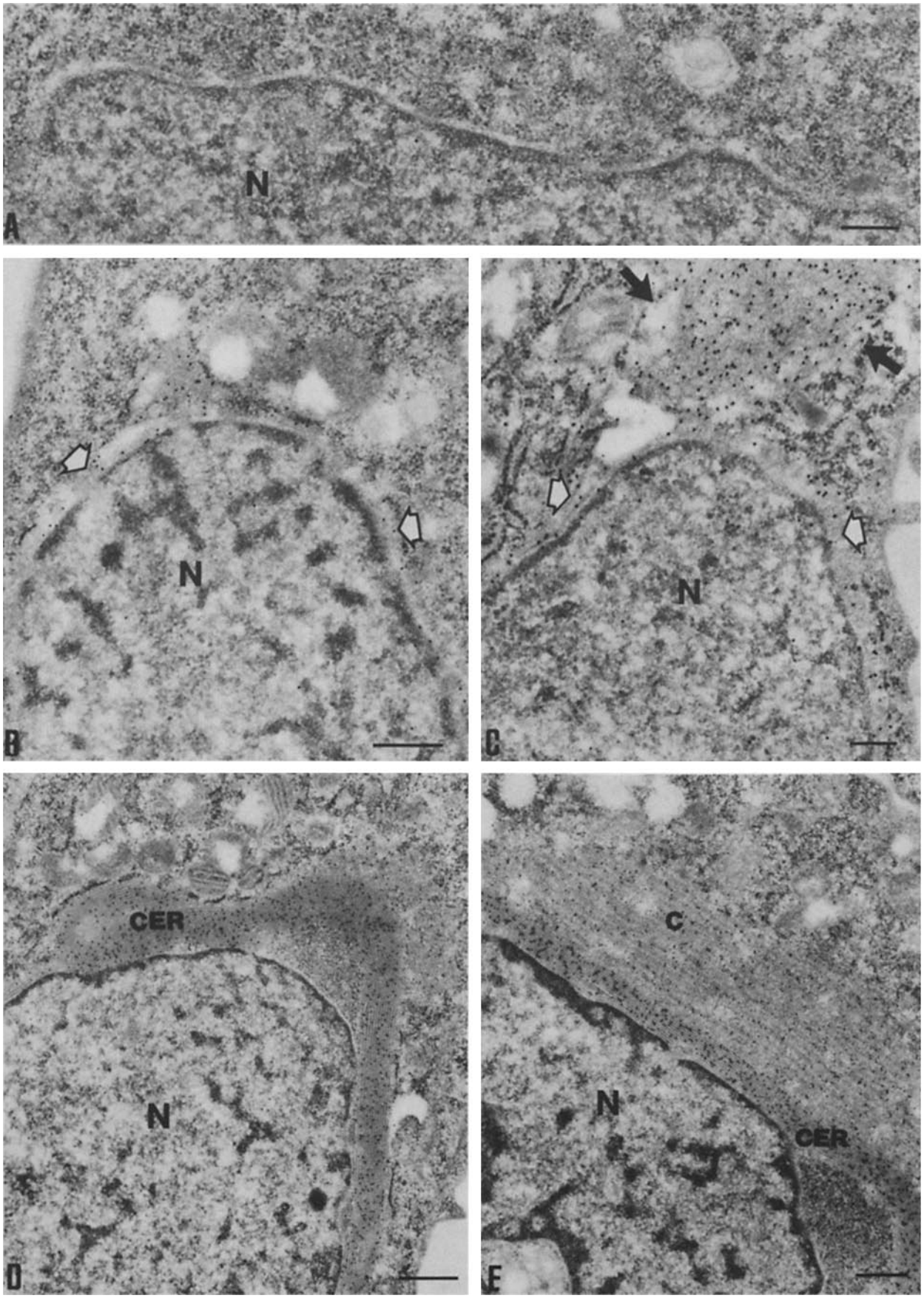


Figure 4. Indirect immunofluorescence localization of HMG CoA reductase in UT-1-cw cells (*A* and *B*) and UT-1 cells (*C*). UT-1-cw cells were grown in culture for 5 d and incubated in the presence of compactin for 0 h (*A*) or 48 h (*B*). UT-1 (*C*) cells were grown in culture for 5 d. Each set of cells was fixed and processed for indirect immunofluorescence localization of HMG CoA reductase as described. Bar, 5 μm.

ization of HMG CoA reductase. Due to the relatively inefficient permeabilization by the saponin detergent used in this procedure, only the outer margins of the crystalloid ER were labeled. In most of the cells examined at this time, there was extensive labeling of membrane around the nuclear envelope.

Fig. 7 is a series of high magnification pictures of the nuclear envelope in compactin-treated UT-1-cw cells. There was no reaction product evident in cells treated with non-immune IgG followed by staining with uranyl acetate and lead citrate to reveal nuclear envelope ultrastructure (Fig. 7*A*). However, in cells treated with anti–HMG CoA reductase IgG but not stained with heavy metal, reaction product was found on the smooth ER membranes next to the nuclear envelope (arrows, Fig. 7*B*) and the outer nuclear membrane (Fig. 7, *B–D*). The outer nuclear membrane could be unequivocally distinguished from the inner nuclear membrane in the region of the nuclear pore where the two membranes are continuous (arrowheads, Fig. 7, *B–D*). Whereas the outer nuclear membrane was heavily labeled, the inner nuclear membrane was devoid of reaction product.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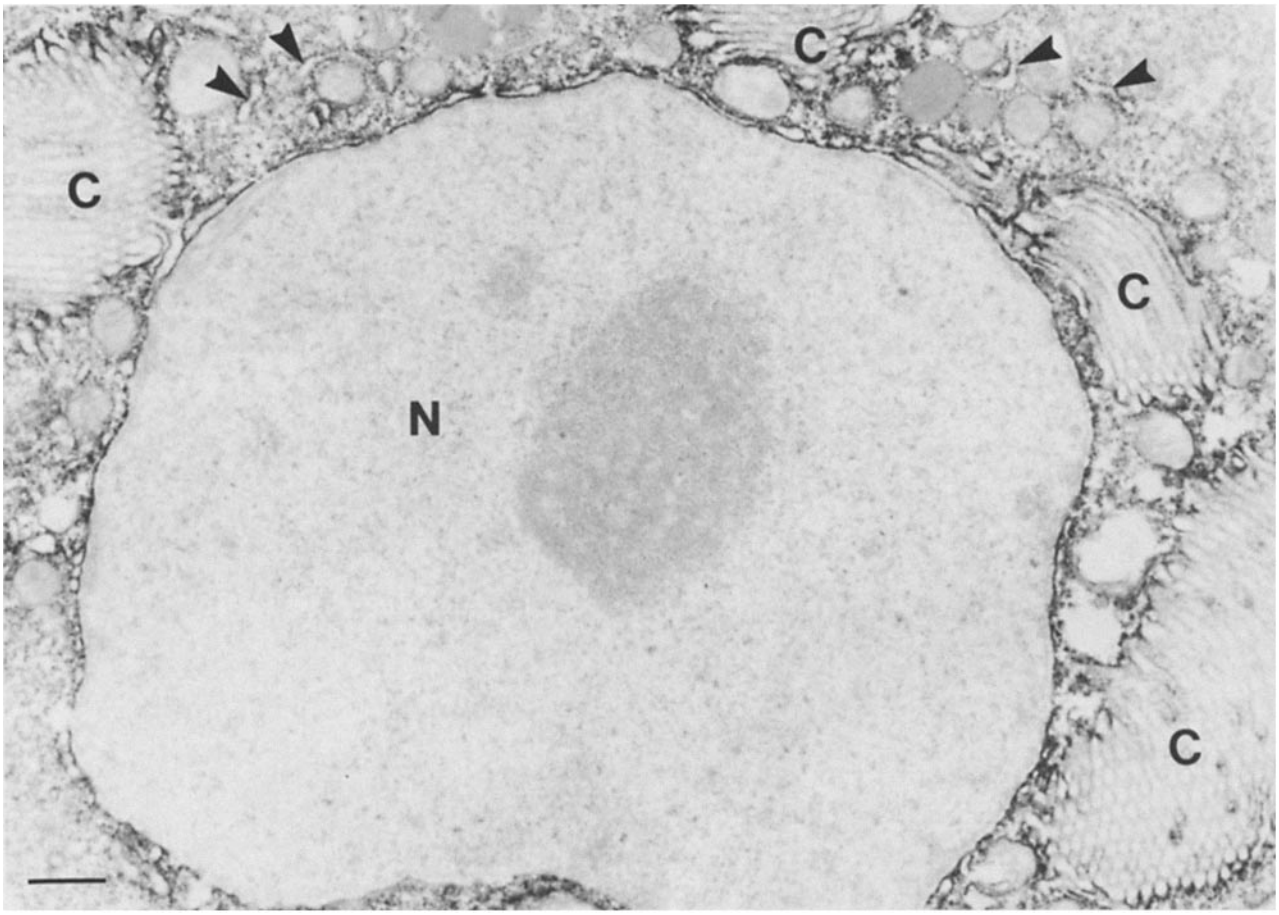

Figure 6. Indirect immunoperoxidase localization of HMG CoA reductase in UT-1-cw cells incubated in the presence of compactin. UT-1-cw cells were grown in the presence of 40 μM compactin for 48 h as described in Fig. 1. Cells were fixed, permeabilized with saponin, and processed for indirect immunoperoxidase immunocytochemistry. Section was not stained with heavy metal stains. *C*, crystalloid ER; *N*, nucleus; arrows, rough ER. Bar, 0.7 μm.

In contrast to the abundant reaction product associated with the nuclear envelope, very little reaction product was found on the rough ER profiles that were scattered in the cytoplasm of the cell (arrows, Fig. 6). Therefore, even with this more sensitive immunocytochemical technique, the rough ER did not appear to contain a significant amount of HMG CoA reductase.

Nuclear Envelope and Associated Smooth ER Contain Newly Synthesized HMG CoA Reductase

If the nuclear membrane and associated smooth ER are the sites of HMG CoA reductase synthesis, then inhibition of protein synthesis should cause the preferential disappearance of HMG CoA reductase from these membranes. To test this hypothesis, UT-1-cw cells were incubated in the presence of compactin for 48 h followed by a 4-h incubation in the presence of cycloheximide to inhibit the synthesis of HMG CoA reductase. The cells were subsequently processed for indirect immunoperoxidase localization of HMG CoA reductase (Fig. 8). Whereas these cells contained abundant reaction product in the crystalloid ER (*C* in Fig. 8), there was an absence of reaction product around the nuclear envelope (compare Fig. 8 with Fig. 6). We also noted that in cycloheximide-treated cells prepared for conventional electron microscopy there were very few smooth ER cisternae near the nuclear envelope (data not shown).

Figure 5. Indirect protein A–gold localization of HMG CoA reductase in UT-1-cw cells at representative stages in the biogenesis of the crystalloid ER. UT-1-cw cells were incubated in the presence of compactin for 0 h (*A*), 24 h (*B*), 48 h (*C*), and 72 h (*D* and *E*) as described in Fig. 1. At each time point, the cells were fixed, embedded in Lowicryl K4M, and processed for protein A–gold localization of anti–HMG CoA reductase IgG binding sites. (*A*) The perinuclear area of a UT-1-cw cell that was not incubated in the presence of compactin. *N*, nucleus. Bar, 0.3 μm. (*B*) The perinuclear area of a UT-1-cw cell that had been incubated in the presence of 40 μM compactin for 24 h. Smooth ER (*arrows*) is labeled with gold. *N*, nucleus. Bar, 0.4 μm. (*C*) The perinuclear area of a UT-1-cw cell that had been incubated in the presence of 40 μM compactin for 48 h. Smooth ER (*open arrows*) and crystalloid ER (*solid arrows*) are labeled with protein A-gold. *N*, nucleus. Bar, 0.3 μm. (*D* and *E*) The perinuclear area of two UT-1-cw cells that were incubated in the presence of 40 μM compactin for 72 h. *N*, nucleus. *CER*, stacks of smooth ER cisternae. *C*, crystalloid ER. Bars, 0.5 μm.

Table I. Effect of Compactin on Anti-HMG CoA Reductase Labeling of Nuclear Envelope-associated Membranes in UT-1-cw Cells

Time of incubation with compactin	HMG CoA reductase activity	Portion of nuclear circumference labeled	
		Less than one-half	Greater than one-half
h	*U/mg*	*% of cells*	
0	1.9	6.0	—
7	1.75	7.0	—
22	10.2	37.0	8.5
48	23.0	41.5	38.0
72	57.0	35.5	57.0

UT-1-cw cells were grown for 5 d and incubated in the presence of 40 μM compactin for the indicated time. At each time point, one set of cells was used to measure HMG CoA reductase activity and one set was processed for indirect protein A–gold labeling of anti-HMG CoA reductase IgG binding sites. 50–200 cells were scored for each time point. Cells with a complete section through the nucleus were examined and tabulated for the presence or absence of gold particles on the circumference of the nuclear envelope. A cell was scored as positive if more than 10 gold particles in a region were present. The results are the average of two separate experiments.

Discussion

Our goal was to determine the sequence of events in the biogenesis of the crystalloid ER. Both the morphological and the immunocytochemical data implicate the outer nuclear membrane as the major, if not exclusive, site of crystalloid ER membrane synthesis. Fig. 2 is a model of how we imagine that new membrane emerges from the nuclear envelope. An important feature of this model is that whereas initially ribosomes synthesize HMG CoA reductase on the outer nuclear membrane, as membrane expansion takes place, HMG CoA reductase synthesis is shifted to the outer membrane of the outermost smooth ER cisternae. In this manner, several layers of smooth ER cisternae often accumulate next to the nucleus.

The membrane of the smooth ER cisternae is the precursor to the crystalloid ER tubules. The flat sheets of the smooth ER cisternae transform into the tubular structure characteristic of the crystalloid ER. The factors that control this shape change are not known. Although the shape of some membranes is controlled by the cytoskeleton (19, 23), we found no evidence that cytoskeletal elements were associated with the crystalloid ER tubules. Most likely, the tubular morphology is due to the intrinsic molecular properties of the membrane. Other investigators have noted that smooth ER can assume both flat and tubular shapes within the same cell (29).

Molecular and biochemical studies indicate that the synthesis and insertion of HMG CoA reductase occurs by a pathway that is common to many membrane proteins (22, 26–28). HMG CoA reductase is synthesized as a 97-kD protein that does not contain a cleaved amino-terminal signal sequence (11). The amino acid sequence of the enzyme contains seven stretches of hydrophobic amino acids that are predicted to span the membrane of the ER seven times and presumably direct the insertion of the protein into the membrane. The membrane insertion of the molecule seems to occur co-translationally and requires the action of a signal recognition particle (7). Although other proteins use similar mechanisms for membrane insertion, the nuclear envelope has not been recognized as a preferred site for the synthesis of these proteins.

At present, little is known about the contribution of the

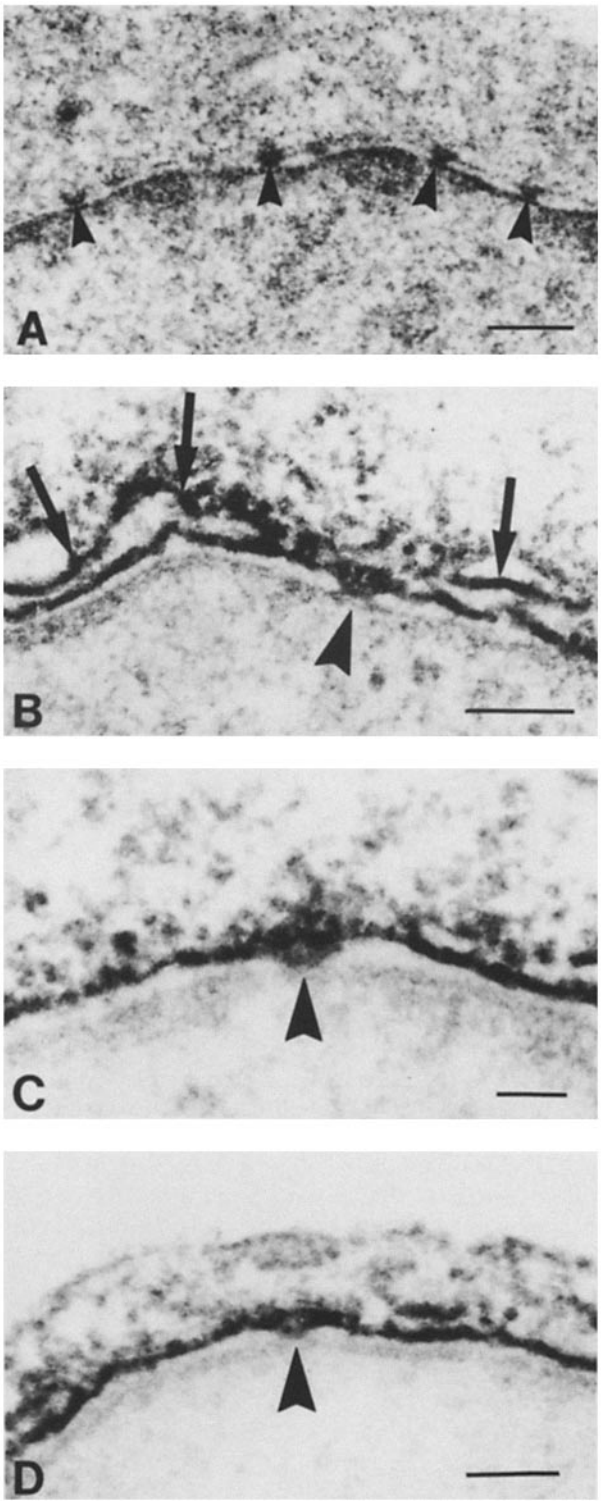

Figure 7. Distribution of HMG CoA reductase around the nuclear envelope of UT-1-cw cells incubated in the presence of compactin. UT-1-cw cells were grown in the presence of 40 μM compactin for 48 h as described in Fig. 1. Cells were fixed, permeabilized with saponin, and incubated in the presence of either nonimmune IgG (*A*) or anti-HMG CoA reductase IgG (*B–D*) before processing for indirect immunoperoxidase localization of IgG binding sites. Sections of nonimmune IgG-treated cells (*A*) were stained with uranyl acetate and lead citrate but anti-HMG CoA reductase IgG treated cells were not stained. *Arrows*, smooth ER; *arrowheads*, nuclear pores. Bars: (*A*) 0.3 μm; (*B*) 0.2 μm; (*C*) 0.1 μm; (*D*) 0.2 μm.

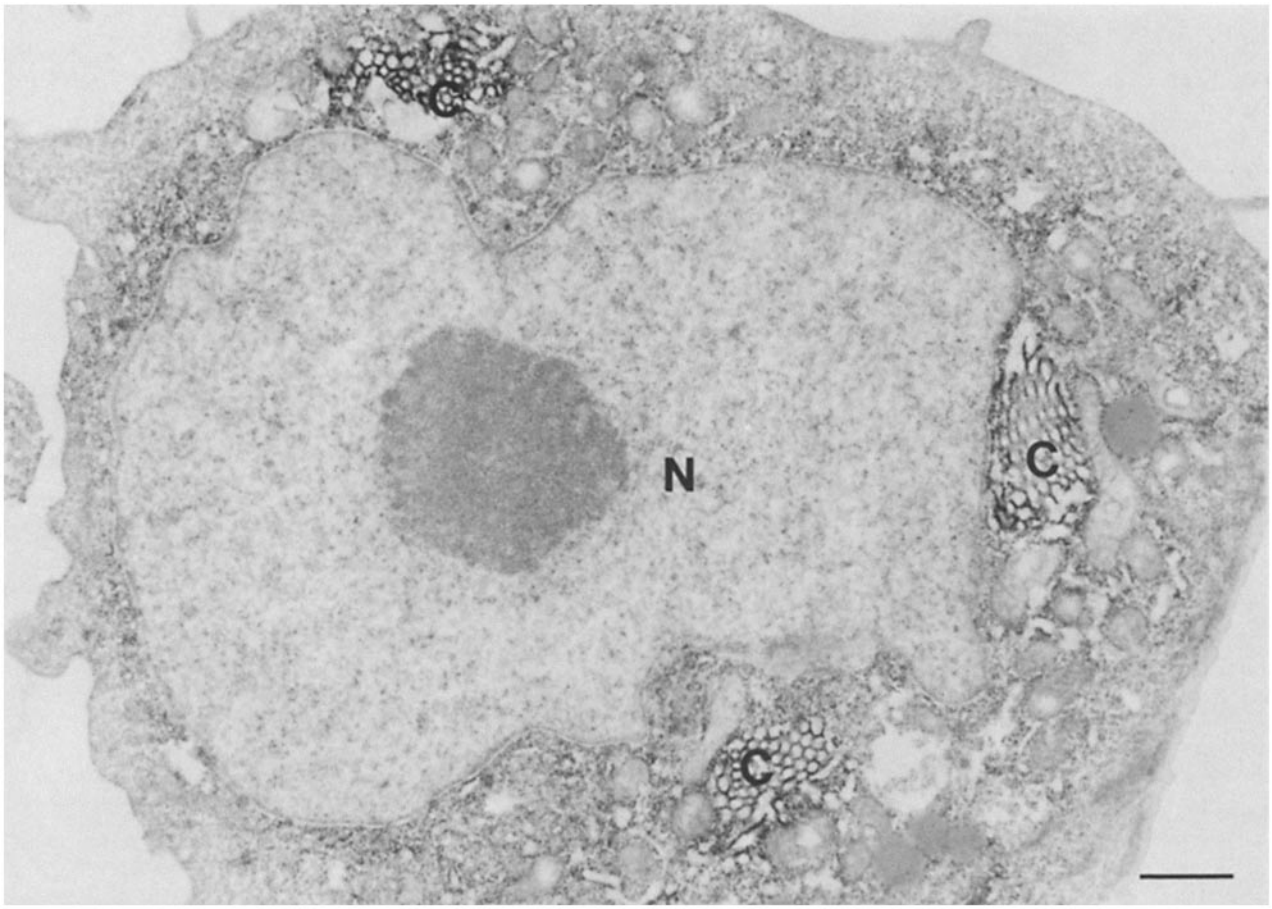

Figure 8. The effect of cycloheximide on the distribution of HMG CoA reductase in UT-1-cw cells incubated in the presence of compactin. UT-1-cw cells were grown in the presence of 40 μM compactin for 48 h followed by an additional incubation in the presence of 200 μM cycloheximide for 4 h. Cells were fixed, permeabilized with saponin, and processed for indirect immunoperoxidase localization of HMG CoA reductase. Section was not stained with heavy metal stains. *C*, crystalloid ER; *N*, nucleus. Bar, 1 μm.

nuclear envelope to the synthesis of membrane proteins (13). Immunocytochemical evidence suggests that another smooth ER membrane protein, cytochrome P_{450}, is synthesized on the outer nuclear membrane (20, 21). Viral membrane proteins in cultured cells infected with enveloped viruses are synthesized on both the outer nuclear membrane and the rough ER (4, 5, 18). The contribution of these two membranes to viral membrane protein synthesis may vary depending on the relative amounts of nuclear membrane and rough ER membrane in each cell type (25).

In UT-1-cw cells, the rough ER occupies from 1 to 4% of the volume density of the cell (24, data not shown). This is a sufficient amount of rough ER to support the synthesis of HMG CoA reductase. However, using two different immunochemical procedures we could not detect significant amounts of HMG CoA reductase associated with this ER. Without more information, we can only speculate that the outer nuclear membrane is chemically specialized for the synthesis of this membrane protein. Indeed, each membrane protein may be synthesized on a specific subset of all the rough ER (including the outer nuclear envelope) in a cell. If this is the case, then the sorting of membrane protein destined for specific compartments in the cell may begin at the level of synthesis and insertion.

The UT-1-cw cell is uniquely suited for studying the biogenesis of membrane as well as the factors that control the synthesis of smooth ER. Unlike cells that are infected with enveloped viruses, the synthesis of large amounts of HMG CoA reductase induces the expansion of the membrane compartment that normally houses this protein. The present study has raised many questions about the synthesis of this membrane. (*a*) What are the signals that cause the mRNA for HMG CoA reductase to synthesize this enzyme preferentially on the outer nuclear membrane? (*b*) How is the phospholipid bilayer expanded to accommodate the insertion of large amounts of HMG CoA reductase? (*c*) What factors control the shape of these membranes? (*d*) Do other integral (or peripheral) proteins reside in this membrane and are they required for its proper form and function? The answers to these and related questions will come with the application of morphological, biochemical, and molecular techniques to the study of this cell. This knowledge should contribute to a broader understanding of how membranes are synthesized.

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