Antibodies to the Golgi Complex and the Rough Endoplasmic Reticulum

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ABSTRACT  Rabbits were immunized with membrane fractions from either the Golgi complex or the rough endoplasmic reticulum (RER) by injection into the popliteal lymph nodes. The antisera were then tested by indirect immunofluorescence on tissue culture cells or frozen, thin sections of tissue. There were many unwanted antibodies to cell components other than the RER or the Golgi complex, and these were removed by suitable absorption steps. These steps were carried out until the pattern of fluorescent labeling was that expected for the Golgi complex or RER. Electron microscopic studies, using immunoperoxidase labeling of normal rat kidney (NRK) cells, showed that the anti-Golgi antibodies labeled the stacks of flattened cisternae that comprise the central feature of the Golgi complex, many of the smooth vesicles around the stacks, and a few coated vesicles. These antibodies were directed, almost entirely, against a single polypeptide with an apparent molecular weight of 135,000. The endoplasmic reticulum (ER) in NRK cells is an extensive, reticular network that pervades the entire cell cytoplasm and includes the nuclear membrane. The anti-RER antibodies labeled this structure alone at the light and electron microscopic levels. They were largely directed against four polypeptides with apparent molecular weights of 29,000, 58,000, 66,000, and 91,000.

Some examples are presented, using immunofluorescence microscopy, where these antibodies have been used to study the Golgi complex and RER under a variety of physiological and experimental conditions. For biochemical studies, these antibodies should prove useful in identifying the origin of isolated membranes, particularly those from organelles such as the Golgi complex, which tend to lose their characteristic morphology during isolation.
peripheral components, most of which were contaminants. This approach minimized the number of contaminants against which antibodies might have been raised.

Step 2: Rabbits were immunized with the purified membrane fractions using a method and schedule of injection, which, in our experience, and that of others, tends to favor the production of antibodies to the major immunogenic components in the sample and not to minor components. This again favored those components present only in the organelle of interest.

Step 3: Unwanted antibodies that were produced during immunization were then removed by suitable absorption steps. In one step, for example, the antisera were treated with those membrane fractions that were usually discarded during the isolation of the organelle of interest. The efficiency with which the unwanted antibodies were removed was rapidly assessed by indirect immunofluorescence labeling of tissue culture cells or frozen, thin sections of tissue. Using this technique it was often possible to determine the nature of unwanted antibodies and then to design suitable absorption steps. A careful choice of cell or tissue favored the preparation of antibodies that were not specific for a particular tissue or even animal. The absorption steps were continued until the pattern and position of fluorescent labeling were characteristic of the Golgi complex or RER.

Step 4: The intracellular structure labeled by the antisera was then determined by electron microscopic studies using immunoperoxidase labeling. The specific proteins labeled by the antibodies were determined by immunoprecipitation.

MATERIALS AND METHODS

Preparation of Antibodies to Golgi and Microsomal Fractions

MICROSOMAL FRACTIONS

Rough microsomes, prepared from dog pancreas (3, 34), were then stripped of ribosomes using EDTA and then washed with high salt (25). These depleted membranes were provided by David Meyer (European Molecular Biology Laboratory [EMBL], Heidelberg, W. Germany).

GOLGI FRACTIONS

Light fractions of Golgi membranes, prepared from rat liver (2), were provided by Jon Green (Biochemistry Department, University of Cambridge, England). The membranes were partially freed of their secretory contents using a modification of the procedures described by Ehrenreich et al. (9). Golgi vesicles (2.6 mg of protein) were suspended in 3 ml of 10 mM Tris-HCl, 10 mM EDTA (pH 8.5), 0.02% (wt/vol) NaN₃, and 40 µg/ml phenylmethylsulfonyl fluoride (PMSF). The mixture was sonicated (Branson sonifier B-12 [Branson Sonic Power Co., Danbury, Conn.]) equipped with a microtip probe) just below the cavitation point for 4-15 s at 0°C. After centrifugation at 100,000 g for 30 min at 4°C, the supernatant was transferred to a 15% (wt/vol) sucrose cushion in HEPES buffer and centrifuged at 100,000 g for 30 min at 4°C. The IgG fraction was then prepared by chromatography on DEAE cellulose (10). The only disadvantage of this procedure was that the resulting rabbit IgG was contaminated by rat IgG present in the original rat plasma. The second procedure avoided this problem. The rat plasma proteins were cross-linked with glutaraldehyde to form a gel (40) and this was used as an immunoadsorbent to remove unwanted antibodies from the rabbit antiserum. Occasionally 2-3 ml of plasma was used to make the immunoadsorbent necessary to treat 1 ml of rabbit antisera to Golgi membranes. An additional advantage of this second procedure was that the immunoadsorbent could be regenerated by treatment with acid and reused several times.

USING MEMBRANE FRACTIONS: The rabbit antisera raised to Golgi membranes were treated with the rough and smooth microsomal fractions (MR+s) normally discarded during the isolation of Golgi fractions (2). Occasionally, the number of unwanted antibodies to plasma membrane components was so high that the amount of plasma membrane in the MR+s fractions was not sufficient to remove them. It was then necessary to introduce an additional absorption step using purified plasma membranes from rat liver (30). For immunofluorescence labeling of certain cells and tissues it was also necessary to treat the antisera with vesicles from intestinal microvilli (23).

The antisera raised to RER was treated with the smooth membrane fraction isolated during the preparation of rough microsomes (3, 34). All membrane fractions were prepared for the absorption steps in the same way. The membranes were suspended in PBS to a concentration of 5-10 mg of protein/ml and treated with an equal volume of 6% (wt/vol) parafomaldehyde, 1% (wt/vol) glutaraldehyde in PBS. After 1 h at room temperature (20°C), the mixture was layered over a 15% (wt/vol) sucrose cushion in PBS containing 50 mM lysine and centrifuged at 100,000 g for 30 min at 4°C. The pellets were resuspended to their original volume in PBS containing 50 mM lysine and left at room temperature for a further 30 min. Triton X-100 was then added, over a 5-minute period, to a final concentration of 0.2% (wt/vol), and the mixture was then layered over a 15% (wt/vol) sucrose cushion in PBS and centrifuged at 100,000 g for 30 min at 4°C. This treatment with Triton X-100 revealed more antigenic sites in the immunoadsorbent, which could then be stored for several months at 4°C in 0.02% (wt/vol) NaN₃ or ideally at −80°C. The absorption steps were carried out by mixing the antisera with the absorbent and leaving this mixture to stand at 1 h at room temperature or overnight at 4°C. The immunoadsorbent bound, unwanted antibodies was then removed by centrifugation at 100,000 g for 30 min at 4°C. The immunoadsorbent could then be regenerated by treatment with acid (40). The amount of immunoadsorbent needed to remove unwanted antibodies depended both on the concentration of these antibodies in the antiserum and on the number of antigenic sites in the immunoadsorbent. Some antigens might be particularly sensitive to the glutaraldehyde used to prepare the immunoadsorbent. In this event, it is possible to fix the proteins with paraformaldehyde alone though the resulting immunoadsorbent is not stable and cannot be regenerated after acid treatment. As a guide to the amounts of immunoadsorbent that might be needed, we treated 1 ml of antisera raised to Golgi membranes with the following amounts of protein needed to prepare the immunoadsorbent: 5-10 mg of Mr=1-2 mg of rat liver plasma membrane, and 1-2 mg of intestinal microvilli. The corresponding experiments using the antisera raised to RER required 2-3 mg of smooth microsomal membrane protein. All of these amounts were determined using indirect immunofluorescence microscopy to monitor the removal of unwanted antibodies (see below). The IgG fraction was then prepared by chromatography on DEAE cellulose (10). Approximately 7-10 mg of IgG was obtained from 1 ml of antisera.

Cells

Normal rat kidney (NRK) cells were provided by Dr. S. J. Singer (University of Louvain, Belgium).
of California at San Diego, La Jolla, Calif.) and were grown in minimum essential medium (MEM) containing 10% (vol/vol) fetal calf serum. Baby hamster kidney (BHK) cells were grown in MEM (Glasgow, Scotland) containing 5% (vol/vol) fetal calf serum. NRK liver cell line, C3, was provided by Ashley Dunn (EMBL, Heidelberg) and was grown in Dulbecco's MEM containing 10% (vol/vol) fetal calf serum. All cells were grown at 37°C in a CO2 incubator (95% air/5% CO2 vol/vol).

**Immunofluorescence Microscopy**

Cells were fixed and labeled with the first antibody (anti-Golgi, anti-RER) using the general procedures described by Ash et al. (1). Frozen, thin sections (0.2-0.5 μm thick) of rat intestinal mucosa and epididymis were prepared as described by Tokuyasu (41), using only formaldehyde as the fixative. Frozen sections attached to glass slides were then labeled with the first antibody as for the cells. The second antibody was a goat anti-rabbit IgG that had been purified using an affinity column containing bound rabbit IgG (40) and then conjugated to rhodamine (5). Wheat germ agglutinin (WGA), conjugated to fluorescein, was obtained from Industrie Biologique Francaise, Paris. For double-labeling experiments the first and second antibodies were passed over a column containing bound WGA to remove IgG molecules that bound to WGA. The fixed, labeled cells and thin sections of tissue were observed using epifluorescence on a Zeiss Photomicroscope III equipped with appropriate filters for rhodamine and fluorescein, and with a 63 x oil-immersion lens.

**Immunoperoxidase Labeling**

This was performed essentially as described by Ohtsuki et al. (27) and Tougaard et al. (42) with the following modifications. NRK cells, grown on cover slips, were fixed with 2% (wt/vol) formaldehyde, 0.05% (vol/vol) glutaraldehyde in 100 mM PBS pH 7.4 for 1 h at room temperature. The cells were then washed three times with PBS and using 50 mM NH4Cl followed by PBS containing 0.2% (wt/vol) gelatin and 0.2% (vol/vol) saponin for 30 min at room temperature. The cells were then treated either with anti-Golgi antibodies (0.2 mg/ml) or anti-RER antibodies (0.2 mg/ml) in PBS containing 0.05% (vol/vol) saponin and 0.2% (wt/vol) gelatin for 30 min at room temperature. The cells were then washed three times with PBS containing 0.05% (wt/vol) saponin and then treated with Fab fragments of sheep anti-rabbit IgG conjugated to horseradish peroxidase (provided by T. Ternynck, Institut Pasteur, Paris) in the same buffer used for the first antibodies. After washing three times with PBS containing 0.05% (wt/vol) saponin, the cells were fixed with 4.5% (wt/vol) glutaraldehyde in 100 mM cacodylate buffer pH 7.2. After washing three times with 100 mM Tris-HCl pH 7.6, the cells were incubated in the medium described by Graham and Karnovsky (16) containing 1 mg/ml dianimobenzidine and 0.001% (vol/vol) H2O2 for 1 h at room temperature in the dark. The cells were then washed with PBS, scraped, and centrifuged (Beckman microfuge; Beckman Instruments, Inc., San Diego, Calif.) for 1 min at room temperature. The pellet was postfixed for 1 h in 2% (wt/vol) OsO4 at 4°C, dehydrated in a graded ethanol series and embedded in Epon. Thin sections were observed using a Zeiss EM/10/A or a Philips 301 electron microscope.

**Antibodies Specific for the Golgi Complex**

**PREPARATION AND SCREENING USING TISSUE CULTURE CELLS:** Light Golgi fractions (2), purified from rat liver, were used. Many of the unwanted secretory contents and absorbed cytoplasmic proteins were removed by sonicating and washing with high salt, and these enriched membrane fractions were then used to immunize rabbits.

NRK cells were used to screen for antibodies to the Golgi complex because these cells are large and flat and therefore very suitable for immunofluorescence studies. They are also derived from a tissue (kidney) different from that used to prepare the Golgi membranes used for immunization (liver). We were thus screening for antibodies that recognized common antigens in the Golgi complex and not those specific for a particular tissue. Tissue culture cells also have the added advantage that their surface can be probed for unwanted antibodies before the cell interior is made accessible to antibodies by mild detergent treatment. In NRK cells, as in many cells grown in tissue culture, the Golgi complex occupies a narrow, crescent-shaped region adjacent to the cell nucleus. Our initial aim, therefore, was to look for fluorescent labeling of the perinuclear region and to remove unwanted antibodies until this region alone was labeled.

When intact, fixed NRK cells were treated with the IgG fraction from a crude rabbit antiserum to Golgi membranes, an intense, nonuniform labeling of the entire cell surface was collected, frozen in small aliquots, and stored at -20°C.
FIGURE 1  Purification of antisera raised to rat liver Golgi fractions, using NRK tissue culture cells. NRK cells, grown on cover slips, were either treated directly with anti-Golgi antibodies (a, b, d, f, and h) or treated after the cell interior was made accessible to the antibodies using Triton X-100 (c, e, g, and i). The second antibody was goat anti-rabbit IgG conjugated to rhodamine. (a-c) IgG fraction of rabbit antisera to Golgi fractions. Note the labeling of the cell surface (a), the extracellular matrix containing fibronectin (b), and the perinuclear region (c). (d and e) After absorption of antisera with MR+s membrane fractions that contain plasma membranes and rough and smooth ER. Cell surface labeling was reduced considerably (d) and the perinuclear labeling was enhanced (e). Note that the extracellular matrix was still labeled (e, arrow). (f and g) After absorption of antisera with rat blood plasma. The cell surface (f) and perinuclear region (g) were still labeled but the extracellular matrix was not. (h and i) After absorption of antisera with both plasma and the MR+s fractions. The cell surface was not labeled (h) and only the perinuclear region within the cell was labeled (i). N, nucleus. × 930.
observed (Fig. 1a). NRK cells secrete large quantities of proteins such as fibronectin, which constitute the extracellular matrix used to attach these fibroblasts to the substratum. The crude rabbit antibodies frequently labeled structures resembling this extracellular matrix (Fig. 1b). When fixed NRK cells were treated briefly with mild detergent before treatment with the antibodies, additional labeling of the cell interior was observed (Fig. 1c). The entire cell cytoplasm was faintly labeled, many vesicles throughout the cytoplasm were labeled, and there was an intense labeling of a region that was usually located on one side of the nucleus. This was a promising sign that at least some of the antibodies in the crude antisera were directed at components in the Golgi complex.

There were two main types of unwanted antibody, those raised to cellular components that contaminated the preparations of Golgi membranes, and those raised to secretory proteins being transported by the Golgi complex from the RER to the cell surface. The first type of unwanted antibody was removed by treating the crude rabbit antisera to Golgi membranes with those membrane fractions that were usually discarded during the preparation of Golgi membranes. These membrane fractions (M_{R+S}) were derived largely from rough and smooth ER and plasma membranes. After absorption with these membrane fractions, the amount of surface and internal labeling was reduced considerably (Fig. 1d and e). The reduction in internal labeling emphasized the labeling in the perinuclear region. It should be noted that this absorption step did not remove unwanted antibodies to secretory proteins, since structures resembling an extracellular matrix were still present (Fig. 1f, arrow).

The second type of unwanted antibody (to secretory proteins) was removed using rat blood plasma for the absorption step, since most of the proteins secreted by rat liver are found in the blood plasma. This treatment did not reduce the labeling of the cell surface (Fig. 1f) but there was no evidence of any labeling of the secreted extracellular matrix. The internal labeling, in the perinuclear region, was slightly reduced compared with that obtained with the untreated rabbit antibodies (cf. Fig. 1g and c, respectively).

When these two absorption steps were combined to remove unwanted antibodies, there was no labeling of the cell surface (Fig. 1h) and the only remaining label in the cell interior was a narrow, crescent-shaped structure adjacent to the nucleus (Fig. 1i). At higher magnifications (Fig. 2a and b), the structure was clearly seen as a reticular network which bore a striking resemblance to the structure first visualized by Camillo Golgi using a silver staining technique (14).

**PREPARATION AND SCREENING USING THIN SECTIONS OF TISSUE:** The enterocytes of rat intestinal mucosa are polarized cells, which, in longitudinal section, reveal a well-defined Golgi complex, just above the cell nucleus (43). When thin, longitudinal sections were treated with the IgG fraction of the antisera to Golgi membranes, the entire cell was labeled with the exception of the nucleus. The brush border, basolateral membranes, and the lamina propria, in particular, were heavily labeled (Fig. 3a). There was no selective labeling of the supranuclear region containing the Golgi complex, in contrast to results obtained with NRK cells at this stage.

After treatment with membrane fractions M_{R+S}, the pattern of labeling changed dramatically (Fig. 3b). The basolateral membranes and the cell cytoplasm were barely labeled, leaving a discrete labeled region just above the nucleus. The brush border and lamina propria were still labeled intensely.

After treatment with blood plasma, the changes were less dramatic though the lamina propria (which contains secreted proteins such as fibronectin) was no longer labeled and there was a reduction in the labeling of the cytoplasm (Fig. 3c).

When both treatments were combined, the Golgi region was labeled but so was the brush border (Fig. 3d). This shows that the choice of absorption steps is, at least in part, empirical, and emphasizes the value of immunofluorescence microscopy for screening the antibodies. The microscopy showed that unwanted antibodies to the brush border were still present and so an absorption step using purified microvilli was needed. When this was done (Fig. 3e), the only region of the cell that was labeled was the Golgi complex, just above the cell nucleus (43). When thin, longitudinal sections of fixed, rat intestinal mucosa, were labeled with anti-Golgi antibodies followed by goat anti-rabbit IgG coupled to rhodamine. (a) IgG fraction of rabbit antisera to Golgi fractions. Note the intense labeling of almost all cellular structures except the nuclei (N). (b) After absorption of antisera with M_{R+S} membrane fractions that contain plasma membranes and rough and smooth ER. The labeling of the supranuclear region became apparent but the brush border (BB) and lamina propria (LP) were still labeled. (c) After absorption of antisera with rat blood plasma containing the secretory products of rat liver. Supranuclear labeling was still apparent and there was no labeling of the lamina propria. (d) After absorption of antisera with both plasma and the M_{R+S} fractions. Supranuclear labeling was apparent but unwanted antibodies to brush border components were still present (e) After absorption of antisera with plasma, M_{R+S} fractions, and purified microvilli. Only the perinuclear region was labeled. (f) The thin section in e was also labeled with WGA conjugated to fluorescein. It labeled the region (G) labeled by the anti-Golgi antibodies as well as the entire plasma membrane, the lamina propria, and the secretory products of the goblet cells (GC). BL basalateral faces. × 1,375.
FIGURE 4 Immunoperoxidase labeling of NRK cells (unstained sections), using anti-Golgi antibodies. (a) An overview of the Golgi complex in NRK cells. Note the labeling of the Golgi cisternae (arrows) and many of the small vesicles (V1) in the Golgi region. Many small vesicles were not labeled (V2), and unlabeled Golgi cisternae (arrowheads) were most often found on one side of the Golgi stacks. There was no labeling of the nuclear membrane (Nm), the RER, the mitochondria (M), and the nuclei (N). The plasma membrane was mostly free of labeling and the only labeling of the cell cytoplasm was that caused by occasional leakage from labeled multivesicular bodies (Mvb). Bar, 0.5 μm. × 22,700. (b) A Golgi complex at higher magnification. In addition to the features pointed out in a, there was the occasional labeling of coated vesicles (CV). Bar, 0.2 μm. × 85,000.

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then labeled was that known to contain the Golgi complex. WGA has been used as a marker for the Golgi complex (44) and it is possible that the anti-Golgi antibodies labeled the Golgi region by binding to those oligosaccharide moieties recognized by WGA. This was not, however, the case. The section labeled with anti-Golgi antibodies and presented in Fig. 3e was also labeled with WGA conjugated to a different fluorescent label, fluorescein (Fig. 3f). In this double-label experiment, WGA clearly labeled the supranuclear region, the brush-border, the lamina propria, and the carbohydrate-rich mucin that comprises the secretory contents of the goblet cells. In contrast (Fig. 3e), the anti-Golgi antibodies only labeled a discrete, supranuclear region that completely enclosed the same region labeled by WGA. If the anti-Golgi antibodies are directed against certain oligosaccharide moieties, they must differ at least in part from those that are recognized by WGA.

Immunoperoxidase studies: By use of immunofluorescence microscopy, the crude antisera to Golgi membranes were freed of unwanted antibodies up to the point where the only part of the NRK cells that was labeled was that known to contain the Golgi complex. The structure labeled by the purified antibodies to the Golgi complex could be ascertainment only by electron microscopic studies. To obtain maximum amplification, we used an immunoperoxidase technique described in the Materials and Methods. The results presented in Fig. 4 show that the anti-Golgi antibodies labeled the membranes of most, but not all, of the Golgi cisternae in any stack and many of the small vesicles in this region, a few of which were coated. Most of the product of the peroxidase reaction was found on the cisternal surface of the membrane and this suggested that the antibodies might have been directed against membrane proteins that had most of their antigenic sites on the cisternal side of the membrane. Many vesicles in the region of the Golgi complex were not labeled and, in general, the Golgi cisternae that were not labeled were usually those on one side of the stack. The ER including the nuclear membrane and the mitochondria were not labeled and neither was the cell cytoplasm nor the nuclear matrix. There was no evidence for any filamentous structure being labeled. The plasma membrane was mostly free of labeling but, very occasionally, small discrete areas were labeled and the intensity of labeling was similar to that seen on Golgi membranes. Further work will be needed to characterize this labeling and to determine whether it is associated with particular structures at the cell surface. The only other structures that were seen to be labeled were multivesicular bodies containing labeled membranes. These may represent Golgi elements that are in the process of being degraded.

In control experiments using normal rabbit IgG, there was no labeling of any cellular structure.

Immunoprecipitation studies: The Golgi vesicles were iodinated by the method of Bolton and Hunter (40), dispersed in RIPA buffer (containing SDS, deoxycholate, and Triton X-100), and treated either with anti-Golgi antibodies, anti-RER antibodies, or control rabbit IgG. A single polypeptide, with an apparent molecular weight of 135,000, was specifically precipitated by the anti-Golgi antibodies (Fig. 5a, lane 2). This polypeptide comigrated with a polypeptide visible in the original extract (lane 1) suggesting it might be a major protein of Golgi membranes. No proteins were precipitated using either anti-RER antibodies (lane 3) or control rabbit IgG (lane 4). NRK cells were grown in the presence of [35S]methionine and extracted with Triton X-100. This extract was treated either with rabbit antibodies to Golgi membranes or with control rabbit IgG. Immunoprecipitations were carried out using either anti-RER antibodies or control rabbit IgG. As a single polypeptide of apparent molecular weight 135,000, precipitated by the anti-Golgi antibodies, comigrated with a polypeptide visible in the original extract (Fig. 5b, lane 1). It should be noted that 200 times the amount of material present in lane 1 was used for each immunoprecipitation experiment and the polypeptide with an apparent molecular weight of 135,000 was not visible in the original cell extract (Fig. 5b, lane 1).

Antibodies specific for the RER

Preparation and screening: Rough microsomes from dog pancreas were freed of ribosomes and peripheral membrane proteins by treatment with high salt and EDTA (see Materials and Methods). This depleted fraction was used to

![Figure 5](image-url) Immunoprecipitation using anti-Golgi antibodies. (a) Iodinated rat liver Golgi vesicles. Lane 1, original rat liver Golgi vesicles; 100 times this amount was used in each immunoprecipitation experiment. Lane 2, anti-Golgi antibodies. Lane 3, anti-RER antibodies. Lane 4, control rabbit IgG. Note that the single polypeptide of apparent molecular weight 135,000, precipitated by the anti-Golgi antibodies, comigrated with a polypeptide visible in the original extract. (b) [35S]Methionine-labeled NRK cells. Lane 1, original extract of NRK cells; 200 times this amount was used in each immunoprecipitation experiment. Lanes 2 and 3, control rabbit IgG in Triton (lane 2) or RIPA (lane 3) buffers. Lanes 4 and 5, anti-Golgi antibodies in Triton (lane 4) or RIPA (lane 5) buffers. The arrowheads on the left indicate major proteins in the original extract that can generally be seen in the control and specific immunoprecipitations.
immunize rabbits and the IgG fraction of the resulting antisera was used to label NRK cells. The antibodies at this stage were surprisingly specific. There was very little labeling of the cell surface, and the cell interior revealed a fine reticular network that spread throughout the cell cytoplasm and an intense labeling of the nuclear membrane (Fig. 6). The cell cytoplasm and nuclear matrix were not labeled. Since this was the pattern we had expected to see there was little need to carry out extensive absorption steps. The low level of surface labeling was removed by treating the crude antibodies with the smooth ER fraction isolated at the same time as RER from the dog pancreas. This absorption step did not affect the pattern of labeling in the cell interior.

In thin, longitudinal sections of rat intestinal mucosa, the purified anti-RER antibodies labeled a reticular network that filled the entire region between the cell nucleus and the terminal web with the exception of one small region just above the nucleus (Fig. 7 a and b). In other thin sections, using the purified, anti-Golgi antibodies (Fig. 7 c and d) it appeared that this supranuclear region was occupied by the Golgi complex.

IMMUNOPEROXIDASE STUDIES: These were performed in essentially the same manner as described above for the anti-Golgi antibodies and were used to determine the structures labeled by these anti-RER antibodies at the electron microscopic level. The results presented in Fig. 8 show exclusive labeling of the ER including the nuclear membrane. Some elements in the Golgi region were labeled but these may represent the transitional elements of the ER. The stacks of Golgi cisternae, the plasma membrane, and the mitochondria were free of label, as were the cell cytoplasm and the nuclear matrix. The label was mainly within the cisternae of the RER, which might suggest that the antigenic sites were located on this side of the membrane.

IMMUNOPRECIPITATION STUDIES: A Triton X-100 extract of [35S]methionine-labeled NRK cells was treated with the purified antibodies to RER essentially as described for the anti-Golgi antibodies. There were four major polypeptides precipitated that were not precipitated by the control rabbit IgG (Fig. 9). Their apparent molecular weights were 29,000, 58,000, 66,000, and 91,000. None of these polypeptides comigrated with major proteins present in the original extract. In contrast to the results obtained with the anti-Golgi antibodies, there were clearly other proteins to which the anti-RER antibodies were directed but the small amounts precipitated either meant that the antigens were minor components of RER or that the antibodies to them had a low affinity.
FIGURE 7  The Golgi complex and RER in rat intestinal mucosa. Thin, longitudinal sections of rat intestinal mucosa were labeled with either anti-RER antibodies (a and b) or anti-Golgi antibodies (c and d) followed by goat anti-rabbit IgG conjugated to rhodamine. By comparing the fluorescence picture (a) with that taken using Nomarski optics (b), the anti-RER antibodies can be seen to label a reticular network largely between the cell nuclei (N) and the terminal web region, with the exception of a small region above the nucleus (arrow) which, in separate experiments (c and d) was seen to be labeled by anti-Golgi antibodies (G). BB, brush border; BL, basolateral faces; GC, goblet cell. × 1,300.

FIGURE 8  Immunoperoxidase labeling of NRK cells (unstained sections), using anti-RER antibodies. The anti-RER antibodies labeled the tubular network of ER throughout the cell cytoplasm and the nuclear membrane (Nm). Ribosomes were difficult to visualize because of the intense staining, and it was not possible to determine whether both the inner and outer nuclear membranes were stained. Mitochondria (M), plasma membranes, the nuclear matrix, and the cell cytoplasm were not labeled. The stacks of Golgi cisternae (inset) were also unlabeled and the elements in this region that were labeled may represent part of the transitional elements (arrowheads) of the ER. Bar 1 μm. × 15,000.
FIGURE 9 Immunoprecipitation using anti-RER antibodies. NRK cells were labeled with [³⁵S]methionine, extracted with Triton X-100, and treated with rabbit IgG or anti-RER antibodies. Lane 1, total extract of NRK cells; 200 times this amount was used in each immunoprecipitation. Lanes 2 and 3, control rabbit IgG in either Triton (lane 2) or RIPA (lane 3) buffers. Lanes 4 and 5, anti-RER antibodies in either Triton (lane 4) or RIPA (lane 5) buffers. The arrowheads on the left indicate major proteins in the original extract that were still generally found to a small extent in the control and specific immunoprecipitations. The arrowheads on the right indicate the four polypeptides that were specifically precipitated by the anti-RER antibodies.

Microscopic Applications for the Antibodies to the Golgi Complex or RER

These antibodies should prove useful in studying the Golgi complex and RER in a variety of physiological and experimental states since they are directed against proteins found in either of these organelles but not to a significant extent in any other. The examples given below illustrate some of these uses and, in most cases, provide further confirmation for their specificity. It should be noted that the antibodies to RER label this structure in all cells that have been tested. In contrast, the anti-Golgi antibodies only label the Golgi complex in cells that originate from rodents. The reasons for this specificity are not yet clear.

THE GOLGI COMPLEX AND RER IN DIFFERENT TISSUE CULTURE CELLS AND TISSUES: The anti-Golgi antisera were raised against Golgi membranes purified from rat liver and the purified antibodies could be used to reveal this structure in thin sections of rat liver (Fig. 10). The Golgi complex in hepatocytes is not restricted to the perinuclear region (9). The antibodies showed that some regions of the complex were...
near to cell nuclei but many were adjacent to the bile capillaries. This scattered distribution of Golgi stacks would have eliminated rat liver as the tissue with which to screen the anti-Golgi antisera.

C3 is an nonmalignant cell line derived from rat liver that exhibits at least some of the polarized features of the native hepatocyte (29). However, labeling with anti-Golgi antibodies clearly showed that the organization of the Golgi complex was not that of the native hepatocyte (cf. Fig. 10), but resembled more that of a typical cultured cell (Fig. 11a). The Golgi complex was a reticular structure, restricted to the perinuclear region, which, on rare occasions, was seen to extend far into the cell cytoplasm. BHK cells (Fig. 11b) were labeled intensely in the perinuclear region.

Cells of the rat epididymis contain an extensive Golgi complex (13). When thin sections of epididymis were labeled with anti-RER antibodies, the entire cell cytoplasm was labeled (Fig. 12a and b) with the exception of the nuclear matrix and large areas in the supranuclear region. When thin sections of epididymis were labeled instead with anti-Golgi antibodies, large areas in the supranuclear region were labeled (Fig. 12c and d). Similar results were also observed for rat intestinal mucosa (Fig. 7) and supports the suggestion that regions occupied by the Golgi complex exclude ER as well as other cellular organelles (46).

**The Effect of Drugs:** High concentrations of Colcemid can severely affect the intracellular transport of secretory proteins and disrupt the organization of microtubules (6). The RER in NRK cells after prolonged treatment with Colcemid appeared as large isolated patches suggesting that the reticular network had become fragmented (Fig. 13a). The nuclear membrane was still intact though parts of it appeared to have the appearance of blebs (Fig. 13a, arrows). The corresponding experiments with the anti-Golgi antibodies showed that the perinuclear region was no longer labeled (Fig. 13b and c). Instead, Golgi elements were seen to underlie the plasma membrane.

Monensin is an ionophore that blocks intracellular transport and is known to cause swelling of Golgi cisternae without affecting the morphology of RER (39). This selective effect could also be observed using the antibodies to RER and Golgi complex on NRK cells. The reticular network of RER was still present after treatment with monensin (Fig. 13d) but the Golgi complex lost its reticular appearance and, instead, large, mostly spherical, vacuoles were observed, which were, however, still restricted to the perinuclear region (Fig. 13e and f).

**The Golgi Complex in Dividing Cells:** An unexpected finding arose during the screening of the anti-Golgi antibodies using NRK cells. Occasionally, an NRK cell was seen to be in the process of division, the example given in Fig. 14 being that of late telophase. The Golgi antibodies labeled what appeared to be a perinuclear region in both daughter cells but, in addition, there was significant labeling of a structure in both daughter cells present at the poles of the remaining mitotic spindle. There have been very few studies on the Golgi complex during cell division at the level of the electron microscope (31; see reference 46). The difficulties in observing this phenomenon must lie largely in finding and identifying Golgi elements that have lost their characteristic morphology. This limitation does not, of course, apply to the anti-Golgi antibodies, though one must always bear in mind the fact that a particular Golgi protein is being followed and not the entire organelle.

**DISCUSSION**

A procedure has been devised to raise antibodies directed specifically against the membrane of either the RER or the Golgi complex. The most important feature of this procedure is that unwanted antibodies, raised to components other than those unique to either RER or the Golgi complex, were removed by suitable absorption steps, the efficacy of these steps being assessed by immunofluorescent techniques. There is every reason to believe that this procedure will be applicable to any cellular organelle or membrane providing that it can be isolated in a reasonably pure state and has a defined location or organization within the cell or tissue used for screening.

The antibodies to Golgi membranes were directed almost entirely against a single polypeptide with an apparent molecular weight of 135,000. The antibodies must also have been directed, to a small extent, against other components found only in the Golgi complex, since the screening procedure removed only unwanted antibodies and there must be many proteins found only in the Golgi complex to which antibodies might be raised. The surprising fact that only one was precipitated suggests that this polypeptide was the most immunogenic protein in rat liver Golgi membranes. In more recent experiments we have shown that the same polypeptide is precipitated

![Figure 11](image-url) The Golgi complex in a rat liver cell line (C3) and BHK cells. Cells were fixed and treated briefly with Triton X-100, followed by anti-Golgi antibodies and goat anti-rabbit IgG conjugated to rhodamine. (a) A rat liver cell line, C3. Note the extensive reticular network that can extend far into the cell cytoplasm. (b) BHK cells. The reticular network of the Golgi complex is restricted to the perinuclear region. N, nucleus. X 800.
The Golgi complex and RER in rat epididymis. Thin sections of epididymis were labeled either with anti-RER antibodies (a and b) or anti-Golgi antibodies (c and d) followed by goat anti-rabbit IgG conjugated to rhodamine. The fluorescence pictures (a and c) are presented together with those taken using Nomarski optics (b and d). Note that the RER filled the entire cell cytoplasm (a), except for large supranuclear regions (unlabeled arrows) that, in separate experiments, were labeled with anti-Golgi antibodies (c). N, nucleus; BL, basolateral faces. x 1,560.

from many rodent cells and also by anti-Golgi antibodies raised in guinea pigs. The Golgi polypeptide would appear to be a membrane protein and, from the pattern of labeling in immunoperoxidase studies, it would appear that most of its antigenic sites are on the cis side of the Golgi membranes. Further work will, however, be needed to determine the topology of this protein in the membrane. In addition, from the pattern of proteins in isolated Golgi membranes, this Golgi polypeptide would also appear to be a major protein. The anti-Golgi antibodies labeled most but not all Golgi cisternae and many smooth vesicles in the Golgi region, a few of which were coated; in other words, they labeled most of those membrane structures that constitute the Golgi complex in NRK cells. The cisternae that were not labeled by the antibodies were usually those on the one side of the Golgi stacks, though whether they were on the cis or trans side could not be determined. This lack of labeling could have resulted from difficulties in making all membranes in the cells equally permeable to antibodies, or it could mean that the antibodies label all but one or two cisternae on one side of the Golgi stack. Further work will be needed to clarify this point. It is particularly intriguing that, very occasionally, discrete areas of the plasma membrane were labeled, suggesting some role for this polypeptide in the transport of material between the Golgi complex and the cell surface. This possibility, however, and a better understanding of the structural features and functional roles of this polypeptide, must await further work. We can only state with certainty that the polypeptide does not resemble either galactosyl transferase or sulfotransferase, since these enzymes have polypeptide molecular weights between 40,000 and 50,000 (12).

The purified antibodies to RER were directed largely against four polypeptides with molecular weights of 29,000, 58,000
FIGURE 13. The effect of monensin and Colcemid on the RER and Golgi complex. NRK cells were treated either with 10 µM Colcemid for 4 h (a–c) or with 10 µM monensin for 1 h (d–f), fixed, treated briefly with Triton X-100 and then with either anti-RER antibodies (a and d) or anti-Golgi antibodies (b, c, e, and f). These were followed by goat anti-rabbit IgG conjugated to rhodamine. In addition to the fluorescence pictures for RER (a and d) and Golgi (b and e), the corresponding pictures for Golgi taken using Nomarski optics are presented (c and f) to emphasize the effect of these drugs on the organization of the Golgi complex. The RER was fragmented by Colcemid (a) but was not affected by monensin (d). Monensin caused the Golgi cisternae to swell (e) but they retained their position near to the nucleus (cf. e and f). The asterisks indicate labeled vesicles (e) that can be seen with Nomarski optics (f). Colcemid caused the fragmented Golgi elements (b) to move from the perinuclear region to the plasma membrane (c, arrows). N, nucleus. x985.

66,000, and 91,000. Other proteins were precipitated but to a much lesser extent. These antibodies labeled exclusively the ER including the nuclear membrane in all cells that were tested. It was not, however, possible to determine whether the antibodies labeled smooth as well as rough ER. The amount of RER in NRK cells is very small and is difficult to see after these cells have been treated with saponin. In addition the product of the peroxidase reaction was so intense that it was difficult to see the ribosomes bound to the ER. From the position of the stain, the antigenic sites would appear to be present mostly on the cisternal side of the membrane though further work will be needed to substantiate this suggestion. Of importance is the fact that the pattern of labeling was distinct from that obtained using the antibodies to Golgi membranes. Here, again, however, the structural features and functional roles of these four polypeptides are unknown as is their relationship to well-characterized microsomal proteins.

The procedure presented here extends the usefulness of the concept of marker enzymes introduced by de Duve and his colleagues (7, 8). This concept arose when cell components from homogenized rat liver were fractionated according to certain physical parameters (sedimentation and, later, density); certain fractions were found to possess almost all of a particular enzyme activity and later electron microscopic studies showed that these fractions often originated from a specific subcellular organelle. The alternative approach that was taken was to isolate an organelle such as the Golgi complex using morphological criteria and then show that the Golgi fractions contained enzymes (e.g., the sugar transferases) found only in low amounts in other membrane fractions (32, 33). The difficulties associated with the application of the concept of marker enzymes are twofold. Firstly, the marker is often not localized...
Golgi complex. It is virtually impossible to isolate an identifiable Golgi complex from cultured cells and many tissues. The Golgi cisternae, for example, are almost always fragmented, giving vesicles whose origin cannot be ascertained. From some tissues (e.g., rat liver) it is possible to isolate identifiable Golgi elements, but the need to preserve morphology for subsequent identification severely restricts the type of purification procedures that can be employed. These difficulties disappear if the isolated fractions of the Golgi complex can be identified using antibodies known to react specifically with this structure in the cell or tissue. As an example, we have used this approach to follow the passage of viral membrane proteins through the Golgi complex (17). BHK-21 cells were infected with Semliki Forest virus and labeled briefly with radiolabeled amino acids or sugars. At different times after labeling, membrane fractions were prepared according to their content of suitable marker enzymes. The viral membrane proteins moved from the RER to the plasma membrane via a smooth membrane fraction. This fraction was initially defined and isolated according to its content of galactosyl transferase, a known marker for the Golgi complex in rat liver (33). There is, however, no direct evidence that this same enzyme is present in the Golgi complex of BHK-21 cells. We therefore used a solid-phase radioimmunoassay to show that this smooth membrane fraction, and no other, specifically bound the anti-Golgi antibodies that label the Golgi complex in BHK cells. Thus the viral membrane proteins were shown to pass through a smooth membrane fraction containing at least some elements of the Golgi complex in BHK cells. In control experiments using membrane fractions from rat liver, we also showed that these antibodies reacted only with the Golgi membrane fractions.

In conclusion, these antibodies allow us to define certain membrane elements of the RER and Golgi complex with more precision than has been possible using morphological criteria. By defining specific membranes antigenically and not morphologically, we can pursue microscopic and biochemical studies that were not previously possible. They should prove particularly useful in our studies on the manner in which the RER and Golgi complex effect the intracellular transport of newly synthesized plasma membrane proteins.

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