Clathrin-immunoreactive sites in the Golgi apparatus are concentrated at the trans pole in polypeptide hormone-secreting cells

(coated cisternae/coated granules/immunocytochemistry/intracellular transport/secretion)

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Communicated by J. E. Rall, April 24, 1985

ABSTRACT By analogy with receptor-mediated endocytosis, clathrin-coated membrane segments at locations other than at the plasma membrane have been implicated in intracellular membrane transport/sorting. The crucial role of the Golgi apparatus in these processes prompted us to study the distribution at this level of clathrin-immunoreactive sites in various polypeptide hormone-secreting cells. With a polyclonal antibody recognizing the polymerized form of clathrin (structural coats or cages), we found that the Golgi area had the highest degree of specific labeling of various cytoplasmic regions, including the subplasmalemmal web. Moreover, the Golgi labeling was not homogeneously distributed, most of the immunoreactive sites being associated with membrane segments at the trans pole. The labeled membranes comprised regions of trans cisternae with and without condensing secretory material, newly formed (maturing) secretory granules freshly released from trans cisternae, and typical coated vesicles. The cis Golgi cisternae, as well as the cytoplasmic region containing transfer (shuttle) vesicles and transitional cisternae of the rough endoplasmic reticulum, were only weakly labeled. Clathrin concentration, degree of assembly of triskelions into coats, duration of clathrin association with membranes, or presence of a coat protein different from clathrin could account for the low immunoreactivity observed at the cis pole as compared to the trans pole.

The migration of secretory polypeptides from their site of synthesis, the rough endoplasmic reticulum (RER), to their site of exocytic release, the plasma membrane, involves a series of processes requiring fusions and fissions of membrane as well as specific targeting (1). By analogy with receptor-mediated endocytosis (2), which is characterized by the association of clathrin coats (3) with the invaginating membrane, a role of membrane carrier/sorter has also been proposed for clathrin at intracellular locations other than the plasma membrane (4). During the intracellular migration of secretory polypeptides, two steps at which membrane interactions are crucial are the transfer of polypeptides from the RER to the cis pole of the Golgi apparatus and their release at the trans pole to yield secretory granules/vesicles (1). To determine the distribution of clathrin at these two sites, we have analyzed, using an affinity-purified polyclonal antibody visualized by the staphylococcal protein A-gold technique, the labeling of the Golgi area of endocrine cells of the islet of Langerhans and of the anterior pituitary.

MATERIAL AND METHODS

Islets of Langerhans were isolated from male SIVZ rats (5), incubated for 3 hr in threshold stimulatory concentration of glucose (1 mg/ml), fixed in sodium phosphate-buffered 2% (vol/vol) glutaraldehyde, pH 7.4, dehydrated, and embedded in Epon (for immunofluorescence) or at low temperature in Lowicryl K4M resin (6) for protein A-gold. Rat anterior pituitaries fixed in situ by aortic perfusion of fixative were processed as described. Clathrin-immunoreactive sites were localized by light microscopy with the indirect immunofluorescence method (7) after removal of the Epon resin (8) and by electron microscopy with the protein A-gold technique (9) on Lowicryl sections, using =15-nm (9) or =6-nm colloidal gold particles (10). For the protein A-gold technique, thin sections were incubated overnight at 4°C on a drop of affinity-purified antibody to clathrin (polyclonal, lot 3-124) diluted 1:100 (5 µg/ml) with phosphate-buffered saline, pH 7.4. After incubation, thin sections were rinsed with distilled water and treated with the protein A-gold solution for 1 hr at room temperature. Sections were washed again with distilled water and stained with uranyl acetate and lead citrate before observation in a Philips EM 300 electron microscope. The specificity of immunostaining was tested by using nonimmune rabbit serum; no labeling was obtained in this condition.

In pancreatic B cells, the quantitative evaluation of the distribution of immunoreactive sites was carried out on photographic prints (×38,000), using an electronic pen connected to a graphic tablet and a microprocessor programmed to calculate the number of points (gold particles) per µm² of the compartment drawn with the pen (see Fig. 24). Four compartments were evaluated separately: (i) subplasmalemmal rim (0.25 µm wide); (ii) cis Golgi region, defined as the cis-most cisterna, the two adjoining ones, and a neighboring cytoplasmic area comprising the transfer (shuttle) vesicles issued from the RER and the transitional cisternae of the latter; (iii) trans Golgi region, defined as the trans-most cisterna, the two adjoining ones, and a surrounding cytoplasmic area including newly formed secretory granules and vesicles neighboring the cisternae; and (iv) nucleus (as cellular background).

The Golgi region of endocrine cells of the anterior pituitary, as well as of islet cells other than B cells, was assessed qualitatively on serial thin sections.

RESULTS

At the light microscope level, indirect immunofluorescence revealed the presence of anti-clathrin binding spots in both

Abbreviation: RER, rough endoplasmic reticulum.
Fig. 1. Immunofluorescence staining, with the affinity-purified polyclonal anti-clathrin antibody, of semi-thin sections of the islet of Langerhans (A) and anterior pituitary (B). In both tissues, the immunofluorescence reaction is visible as bright spots in the vicinity of the unreactive cell nucleus (asterisk). The crescent shape (arrows) as well as the location of the fluorescent spots suggest that they correspond to Golgi regions of secretory cells. (Epon embedding without osmium tetroxide postfixation; both ×840.)

islet and anterior pituitary cells. The fluorescence was particularly abundant in the juxtanuclear area and often showed a crescent shape (Fig. 1). Both the localization and shape of the immunofluorescence reaction suggested that Golgi regions of islet cells were stained. At the electron microscope level, the immunoreactivity revealed by colloidal gold particles appeared to be of low intensity throughout the cytoplasm except in the Golgi region and, to a lesser extent, in the subplasmalemmal area (Fig. 2A). The quantitative values of clathrin immunolabeling per μm² of the nuclear, cis and trans Golgi, and subplasmalemmal compartments of insulin-containing cells are shown in Table 1. In the Golgi region, a marked asymmetry of the immunolabeling was observed between the cis and trans poles of the complex. At the trans Golgi pole, there were numerous gold particles that appeared to be preferentially associated with extensive segments (<6 μm) of cisternae with or without condensing secretory material, and with vesicles and newly formed secretory granules (Fig. 2 A–D). The coated membranes on these compartments are not optimally demonstrated on Lowicryl-embedded material needed for clathrin immunolabeling, but these compartments were previously recognized as "coated" in conventional nonimmunolabeled Epon thin sections (12, 13). At the cis pole, the low degree of immunoreactivity was distributed over cisternae and the cytoplasmic area between these and the RER. In islet cell types other than B cells—e.g., glucagon, somatostatin, and pancreatic polypeptide cells—as well as in endocrine cells of the anterior pituitary, the marked asymmetry of clathrin immunolabeling of the Golgi region was also evident (Figs. 2 E and F and 3 A and B). Fig. 3 A and B shows an example of the extensive coating of maturing secretory granules in the Golgi area of prolactin cells, as evidenced on serial sections.

DISCUSSION

The current hypothesis concerning the role of intracellular nonplasmalemmal clathrin coats (4), as well as the analysis of the transport of a viral protein showing two waves of clathrin-coated vesicles along the RER–plasma membrane route (14, 15), suggests that membrane interactions at both the cis and trans poles of the Golgi apparatus involve clathrin. The results of the present study indicate that a marked level of clathrin immunoreactivity is detected only at the trans Golgi region. The previous immunocytochemical characterization (11) of our polyclonal antibody to clathrin showed that it recognizes clathrin polymerized into coats rather than the "soluble" pool of clathrin, presumably in the form of triskelions (16). Although the Lowicryl embedding needed to demonstrate clathrin immunoreactive sites at the ultrastructural level does not permit optimal visualization of coats or cages, it is assumed in the following discussion that anti-clathrin-rich sites on immunostained sections correspond most likely to regions underlined with structural coats. At the cis Golgi pole, microvesicles that are thought to mediate the RER–Golgi transfer of secretory and membrane proteins (1) reside in a cytosolic matrix with a microfibrillar component. Typical clathrin coats are not seen in association with such vesicles. The immunocytochemical data would not exclude, however, the presence in this region of triskelions of clathrin. Alternatively, and this by analogy with the formation of smooth endosomes from clathrin-coated pits (17), one could envisage that the association of a clathrin coat with transfer vesicles is a more rapid phenomenon than at the trans pole. For example, the role of clathrin coats at this level could be restricted to the step of fission from transitional elements of the RER followed by the rapid dissociation of the coats. It should be pointed out, however, that the identification of bona fide clathrin coats on transitional elements has remained elusive (18). Other possibilities that must be envisaged to account for the low labeling at the cis pole are that only a limited number of clathrin-coated vesicles are sufficient to mediate the RER–Golgi transfer, that clathrin coats departing from the classical pattern (16, 19) are involved, or that a coat protein different from clathrin is concerned. These alternatives cannot be resolved at present, nor do we know whether individual clathrin triskelions, without forming a

Table 1. Clathrin immunolabeling of various compartments of the B cell

<table>
<thead>
<tr>
<th>Compartment</th>
<th>No. of gold particles per μm²</th>
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<tbody>
<tr>
<td>Subplasmalemmal rim</td>
<td>3.34 ± 0.32</td>
</tr>
<tr>
<td>cis Golgi</td>
<td>3.46 ± 0.41</td>
</tr>
<tr>
<td>trans Golgi</td>
<td>26.52 ± 1.10</td>
</tr>
<tr>
<td>Nucleus</td>
<td>0.40 ± 0.11</td>
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</tbody>
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For the delineation of the various compartments, see Material and Methods and Fig. 2A. Values are means ± SEM. P values were determined with Student's t test: subplasmalemmal rim versus cis Golgi, not significant; cis Golgi versus trans Golgi, P < 0.001; trans Golgi versus nucleus, P < 0.001; subplasmalemmal rim and cis Golgi versus nucleus, P < 0.001. Eleven islets were evaluated. In each islet, 6–10 B cells were examined; a total of 2183 gold particles in 777 μm² were counted. Gold particles ~ 6 nm (10).

Fig. 2 (on following page). (A) Thin section of B cells immunostained with anti-clathrin antibody revealed by the protein A–gold technique. The field comprises the three areas (delimited here with vertical bars) in which the immunolabeling was quantitated. From top to bottom: subplasmalemmal rim (spr), cis Golgi region, trans Golgi region. The low degree of immunolabeling of the subplasmalemmal rim and cis Golgi
region can be compared with the marked immunoreactivity of the trans region. (Lowicryl embedding; gold particles ~ 15 nm; ×34,000.) (B) Thin section of a Golgi area showing the asymmetrical distribution of clathrin immunoreactivity across the Golgi apparatus. While the cis region has a low degree of immunostaining, numerous gold particles appear on the trans region, where they are associated with the limiting membrane of both cisternae (arrows) and newly formed secretory granules (asterisks). (Lowicryl embedding; gold particles ~ 6 nm; ×47,000.) (C) Gold particles revealing clathrin immunoreactive sites decorate the membrane of extensive segments of trans Golgi cisternae, as well as of numerous vesicles (arrows). (Lowicryl embedding; gold particles ~ 6 nm; ×38,000.) (D) Detail of a maturing secretory granule with extensive clathrin immunolabeling of the limiting membrane (Lowicryl embedding; gold particles ~ 6 nm; ×49,000.) (E) Golgi area in a glucagon-containing A cell of the islet of Langerhans. The cis–trans asymmetry of the clathrin immunostaining is evident, with most gold particles concentrated on elements at the trans pole, including a newly formed (maturing) secretory granule (arrow). (Lowicryl embedding; gold particles ~ 6 nm; ×31,000.) (F) Clathrin immunolabeling of several secretory granules in the Golgi area of an A cell. (Lowicryl embedding; gold particles ~ 15 nm; ×42,000.)
FIG. 3. (A and B) Two consecutive serial sections of the Golgi area of a prolactin-containing cell showing the characteristic polymorphous shapes of maturing secretory granules. The serial sections allow the identification of the extensive coating (indicated by broken lines) of the membrane on the same granule. (Osmium tetroxide postfixation; Epon embedding; ×42,000.) (Inset) Clathrin immunolabeling of several maturing granules. (Lowicryl embedding; gold particles ~ 6 nm; ×56,000.) (C) Golgi area of a somatotroph cell after clathrin immunostaining. The asymmetrical distribution of clathrin immunoreactive sites across the Golgi apparatus is evident, with the marked labeling of the periphery of maturing secretory granules (asterisks) at the trans pole. (Lowicryl embedding; gold particles ~ 6 nm; ×35,000.) (Inset) Clathrin immunolabeling of the limiting membrane of a maturing secretory granule in a somatotroph cell. (Lowicryl embedding; gold particles ~ 6 nm; ×41,000.)
recognizable coat, may mediate some sort of intracellular mobilization of membranes. If, however, as for the plasma membrane step, one takes typical clathrin coats as the morphologic markers of what we propose to call "receptor-mediated, nonplasmalemmal intracellular transport," the present results would indicate that RER-Golgi transport, possibly including cis-trans migration, is not comparable to trans Golgi–plasma membrane transport.

The asymmetrical clathrin immunolabeling of Golgi membranes can be viewed as an additional expression of heterogeneity across the Golgi complex, together with previously evidenced changes of intramembrane particle and filipin–sterol complex concentration (for review, see ref. 13), enzymes (for review see ref. 22), or antigens—e.g., the 135-kDa protein (23). Besides their postulated role in membrane interactions (4), clathrin-coated vesicles were shown to acidify the vesicle content in vitro (24, 25). Isolated Golgi membranes also contain proton pumps (26) and, in the case of pancreatic B cells, the relationship between the trans coated compartment and the conversion of proinsulin seems interesting: a clathrin-coated Golgi-related compartment has recently been shown to be linked to the conversion of proinsulin (27, 28), and a proinsulin converting enzyme was identified as a thiol protease with an acidic pH optimum (29).

The coated compartment of B cells (and of other polypeptide hormone-secreting cells where precursor/product conversion occurs) may be a critically acid compartment involved in the proteolytic processing of prohormones.

2The best characterized system in this respect is that involving mannose 6-phosphate receptors for lysosomal enzymes (for review see ref. 20); recent immunocytochemical data also suggest the presence of (pro)insulin binding sites on membranes of the Golgi apparatus in B cells (21).

We thank A.-M. Lucini, G. Perrelet, and G. Tripet for technical assistance, G. Negro and P.-A. Rüttimann for photographic work, and I. Bernard for typing the manuscript. This work was supported by Grant 3.460.83 from the Swiss National Science Foundation.