Macrophage membranes and clathrin

J. Aggeler, R. Takemura, B.A. Nichols, and Z. Werb

Introduction

Clathrin-coated pits and vesicles were first described in detail by Roth and Porter (1, 2) in the mosquito oocyte, and their role in adsorptive endocytosis suggested. In the intervening twenty years, the molecular structure of the clathrin coat has been elucidated, and a number of functions for clathrin-coated structures have been proposed. Although the structure of the clathrin basketwork is now understood in some detail, the exact mechanism(s) of assembly and vesicle recognition are still largely unknown.

There are at least two general ways to approach the problem of clathrin function in cells. The first is to define the functions of coated vesicles within cells (Fig. 1). Studies in a wide variety of cell types have confirmed the important role of coated vesicles in receptor-mediated endocytosis of soluble ligands (3–11), and recent studies in our laboratory and others have indicated that clathrin basketworks are also present during phagocytosis of various particles by macrophages (12–14), a specialized type of adsorptive endocytosis. In addition to their role in endocytosis, coated vesicles also function during membrane retrieval in secretory cells (membrane recycling) (15, 16) and as transcellular shuttles in certain epithelia (17). Although not all secretory vesicles are coated, cortical coated vesicles containing secretory products have been described in several cell types (16, 18), including macrophages (19, 20). In addition, it has been suggested that coated vesicles mediate intracellular membrane shuttling, especially between the rough endoplasmic reticulum and the Golgi apparatus and among Golgi compartments (21).

A second way to approach the role of clathrin in cells is to ask how the coat itself functions; several possibilities have been suggested. Bretscher et al. (22) have suggested that coated pits act as molecular filters, capturing certain plasma membrane receptors, either occupied or unoccupied, and sorting these membrane proteins. Heuser (23) and Harrison and Kirchhausen (24) have suggested that clathrin coats have a biomechanical role in the formation of coated vesicles and their pinching off from the plasma membrane. In addition, the possibility of targeting of coated vesicles within cells has been discussed (21), although it seems unlikely that the coats themselves possess sufficient specificity in light of the highly conserved nature of the clathrin molecule (9). It is at present not known which, if any, of these functions is biologically important.

The macrophage has proved to be an excellent model cell type for studying both the role of clathrin-coated vesicles in the membrane economy of cells and the biochemistry of clathrin coat assembly. The techniques used for culture of mouse peritoneal macrophages (12, 25), for spreading onto immune complexes (26–28), for preparation of replicas of broken-open cells (12, 23, 25), and for cytochemistry (19, 29) and thin-section transmission electron microscopy (12) were as previously described.

Results and discussion

Presence of coated vesicles in macrophages

Endocytosis via clathrin-coated pits and vesicles in macrophages (Kupffer cells) was first noted in 1962 by Roth and Porter (1). Since that time many studies have indicated that clathrin-coated vesicles are involved in adsorptive endocytosis in macrophages (30–34), and a variety of tracers, including ferritin
Fig. 1. Coated vesicle functions in macrophages. Receptor-mediated endocytosis in macrophages follows a pathway from the membrane surface through clathrin-coated pits (CP) and vesicles (CV) into an intermediate noncoated endosome (En) compartment in which membrane and content are sorted for ultimate delivery to the plasma membrane (recycling) or to secondary lysosomes (2°Ly). Phagocytosis of latex beads (LB) and of immunoglobulin-coated erythrocytes (E IgG) also involves clathrin basketwork, but it is not known whether phagosomes traverse an endosome-like compartment on their way to lysosomes. Both small (50–70 nm) and large (150–200 nm) coated vesicles are frequently observed budding off the edges of flattened cisternae and tubular structures in the Golgi-GERL of macrophages. At least some of these coated vesicles are primary lysosomes (1°Ly) destined to fuse with coated vesicles and endosomes. Both coated vesicles and large flat patches of clathrin basketwork are abundant on the adherent surfaces of macrophages. These coated areas may result from membrane shuttling during cell spreading and/or as a response to specific receptor-ligand interactions at these surfaces.

(31, 32), colloidal gold (30, 34), and others (34), have been shown to enter these cells in coated vesicles. Our observations using indirect immunofluorescence (28), platinum replicas, and thin-section electron microscopy (Fig. 2) confirm that clathrin-coated vesicles are at least as abundant at the plasma membrane surface of these actively endocytic cells as they are in other cell types. It has been estimated that about 2 per cent of the plasma membrane surface of many cell types is coated (3, 10, 11, 23), and our measurements from replicas of broken-open macrophages indicate that 1.3 per cent of the upper plasma membrane surface and 3.5 per cent of the adherent surface of cells cultured on glass coverslips are covered with clathrin basket-works (Table I). It has been established for a variety of receptors in other cell types (3, 6, 8, 9, 11) that cortical coated pits and vesicles are primarily involved in specific receptor-mediated endocytosis. Recent studies with galactose-conjugated colloidal gold (35) and with horseradish peroxidase (HRP) (Fig. 3) (19), which is taken up by the macrophage mannose/N-acetylglucosamine receptor (36), have indicated that this is probably the case in macrophages also. Steinman et al. (37), in their classic study of membrane recycling during pinocytosis, estimated that macrophages turn over their plasma membrane every 33 min. More recently (38), however, these investigators have stated that their original experiments were carried out in such a way that the rapid formation of endocytic coated pits and vesicles was missed. Sung et al. (36) have reassessed the use of HRP as a bulk phase marker in these studies and concluded that 30 to 50 per cent of HRP uptake is actually receptor-mediated (i.e., probably via coated vesicles). The consequences of this finding on the original estimate of membrane flow in macrophages are not entirely clear. In fibroblasts, it has been estimated that the uptake of fluid phase markers can be completely accounted for by coated vesicles (8). Both the fact that macrophages can internalize HRP three to four times faster than fibroblasts (37) and the fact that at least 50 per cent of HRP uptake by macrophages is not receptor-mediated (36) suggest that these cells probably have other mechanism(s) for fluid phase pinocytosis, for example, trapping of solute within vesicles formed by collapsed membrane ruffles or folds.

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<th>Table I. Extent of clathrin basketworks on macrophage plasma membranes.</th>
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<td>Frequency of basketworks (number/μm²)</td>
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<td>Free upper surface</td>
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<td>Adherent ventral surface</td>
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