GERERAL DISCUSSION

In the present study, I investigated the domain function of clathrin adaptor proteins to explore the mechanism regulating the function of clathrin-coated vesicles. The summary was shown in Fig. IV. In CHAPTERs I and II, I described the interaction between the γ1-adaptin ear domain and Rabaptin-5. To date, several proteins that bind to both the ear domain of γ1-adaptin and related GAE domains of GGAs have been identified. Sequence comparison of these proteins has uncovered the conserved γ1 ear-binding motif with a general consensus sequence, DFxxΦ (Φ is a hydrophobic residues), which is named acidic phenylalanine motif (139). The X-ray crystallographic structures of the GAE domains in complex with a DFxxΦ-containing peptide have recently been determined; the GGA1 GAE domain in complex with a peptide from the p56 accessory protein, and the GGA3 GAE domain with a peptide from Rabaptin-5 (144, 145). These studies revealed that the binding site of both GAE domains consists of a basic residues cluster and a shallow hydrophobic pocket, which I predicted using the ear domain of γ1-adaptin in CHAPTER II. However, unexpectedly, the conserved basic residue participates in the recruitment of the accessory proteins not by their charges but rather by their hydrophobic portions of the side chains. Conserved acidic residues in the acidic phenylalanine motif do not form stable contacts with the GAE domains. However, acidic residues seem to be preferred before the phenylalanine. Therefore, it is conceivable that having an acidic side chain produces a nonspecific electrostatic effect in solution.

Recently, the hinge region of GGAs has also been reported to bind to the γ1-adaptin ear domain (140). Therefore, it is possible that GGAs might be accessory proteins of AP-1. It is an interesting issue to determine if GGAs are accessory proteins of AP-1 or itself clathrin adaptors. Several lines of evidence support the idea that GGAs are accessory proteins of AP-1. Doray *et al.* showed the colocalization of stably transfected GGA2 and endogenous γ1-

adaptin on coated buds of the TGN by immunoelectron microscopy (140). Moreover, mutant MPR that does not bind GGAs fails to enter AP-1-coated vesicles. These results suggested that AP-1 and GGAs might interact and cooperate in the same sorting step. Furthermore, there has been no evidence that GGA proteins are stable components of clathrin-coated vesicles in mammalian cells so far. Instead they redistribute very quickly to the cytoplasm under conditions where AP-1 stays on the membranes (141). These results suggest that GGA proteins are not necessarily packaged into vesicles but rather help recruit coat components and cargo into budding vesicles. The studies of accessory proteins of AP-2 also supported this idea. Many accessory proteins that bind the α-adaptin ear domain can recognize their specific cargo and clathrin, and collaborate with AP-2 to promote optimal clathrin recruitment and assembly (25). Accessory proteins of AP-2 expand the sorting repertoire of the coat rather than generating separate classes of transport vesicle. These results support the possibility that GGAs are accessory proteins of AP-1.

However, these results do not exclude the possibility that the GGAs also nucleate their own vesicles. In triple labeling of γ1-adaptin, GGA1 and clathrin in immunofluorescence, there were a number of structures that were positive for GGA1 and clathrin but not for γ1-adaptin, and for γ1-adaptin and clathrin but not for GGA1, although these staining patterns were similar (141). The result suggests that two populations of clathrin-coated vesicles bud from the TGN; one with clathrin and GGA1 and one with clathrin and AP-1. Another reports from yeast have demonstrated that AP-1 and GGAs facilitate the parallel pathway (82), and it has been proposed that clathrin/AP-1-coated vesicles act in a TGN-to-early endosome pathway, whereas clathrin/GGAs in a direct TGN-to-prevacuolar compartment (equivalent to mammalian late endosome) pathway (142, 143). In addition, I did not observe any enhancement of γ1-adaptin staining at the TGN in GGA-overexpressing cells, but rather observed that the staining was excluded from GGA-localizing areas

(data not shown). If GGAs help recruitment of AP-1, overexpression of full-length GGAs should have enhanced the recruitment of γ1-adaptin to membranes. At least an increase in colocalization of γ1-adaptin and GGAs should have been observed if they are in a same vesicle. These results suggest that GGAs also nucleate their own vesicles.

The discovery and characterization of GGAs have greatly advanced the understanding of protein sorting at the TGN, especially clathrin/AP-1-mediated protein sorting. However, sorting regulation by accessory proteins of AP-1 is still in chaos. Several accessory proteins including Rabaptin-5 have similar FxxΦ motif, suggesting that these proteins bind to the same region of the ear domain of γ1-adaptin. They could bind to each AP-1 in the same vesicle, or bind to AP-1 sequentially. What determines the order of the binding? Although the homology of the ear domains of γ1-adaptin and GGAs suggests a similar function of the domains in AP-1- and GGA- coated vesicles, are there any preferences of accessory proteins in binding to AP-1 and GGAs? If the cargos of GGAs incorporate into AP-1-coated vesicles, are ubiquitinated proteins also transported by AP-1? Can AP-1 form its own vesicle without GGAs?

A future challenge will be to answer these new questions and clarify the complicated protein network of membrane trafficking.

FIGURES

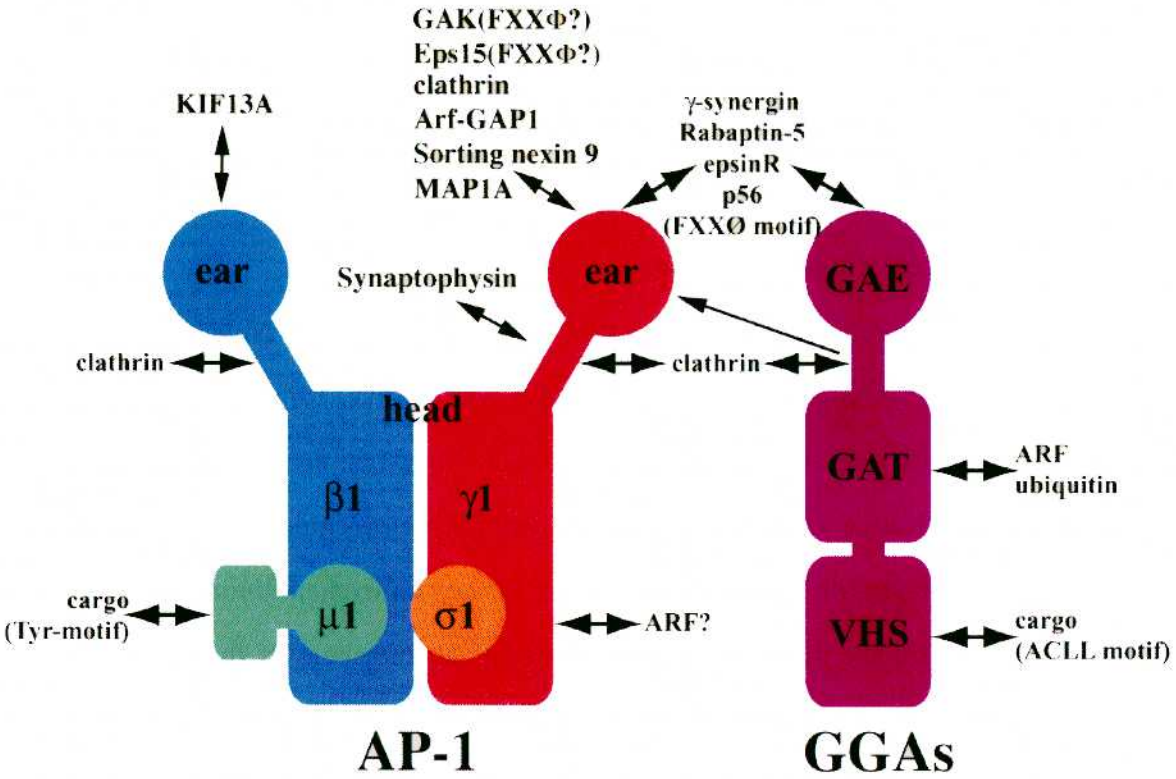

Fig. IV. The summary of the binding proteins of AP-1 and GGAs

The binding partners of AP-1 and GGAs identified so far were shown. The interaction between the ear domain and Rabaptin-5 and between GAT domain and ubiquitin were characterized in this work.