ABSTRACT

AP-1 and GGAs (Golgi-localizing, γ-adaptin ear domain homology, ADP-ribosylation factor (ARF)-binding proteins) are adaptor proteins of clathrin-coated vesicles that mediate transport between the *trans*-Golgi network (TGN) and endosomes. To explore the regulatory mechanisms underlying these transport events, I search for binding proteins of AP-1 and GGAs by yeast two-hybrid screening, and identified Rabaptin-5 and ubiquitin, respectively, as their binding partners. In CHAPTER I, I provide the first evidence for the interaction between γ1-adaptin, a subunit of the AP-1 adaptor complex, and Rabaptin-5. Rabaptin-5 is an effector of the small GTPases, Rab5 and Rab4, and regulates membrane docking with endosomes. A further two-hybrid analysis revealed that the interaction occurs between the ear domain of γ1-adaptin and the COOH-terminal coiled-coil region of Rabaptin-5. A subsequent pull down assay showed that this binding is not affected by Rabs. Coimmunoprecipitation and immunocytochemical analyses showed that the interaction also occurs *in vivo*, and γ1-adaptin and Rabaptin-5 significantly colocalize probably on recycling endosomes. These results suggest that the γ1-adaptin-Rabaptin-5 interaction may play a crucial role in docking of clathrin-coated vesicles derived from the TGN with endosomes or of those from endosomes with the TGN, and raises a possibility that AP-1 can function not only on the TGN but also on recycling endosomes. In CHAPTER II, by collaborating with Wakatsuki and colleagues, I report the structure of the γ1-adaptin ear domain by X-ray crystallography and define the binding site for Rabaptin-5 by two-hybrid and biochemical analyses. The human γ1-adaptin ear domain consists solely of an immunoglobulin-like fold, unlike the ear domain of α-adaptin, a subunit of another adaptor protein of clathrin-coated vesicles. Structure-based mutational analyses revealed a binding site for Rabaptin-5 that is composed of conserved basic residues, indicating that the mechanism underlying recruitment of accessory proteins by γ1-adaptin is

distinct from that by α-adaptin. In CHAPTER III, I examined the interaction between GGAs and ubiquitin. Recent studies have shown that ubiquitin modification can serve as a strong signal for both endocytosis and lysosomal targeting. Many regulatory proteins containing ubiquitin-binding modules function in these sorting processes. Using pull down assays, I showed that the GAT (GGA and Tom1) domains of GGAs interact with ubiquitin. The GAT domains have previously been shown to interact with GTP-bound ARF and to be crucial for membrane recruitment of GGAs. A further analysis showed that the C-terminal subdomain (C-GAT) of the GAT domain, which is distinct from the N-GAT subdomain responsible for ARF binding, binds to ubiquitin. The binding is mediated by interactions between residues on one side of the α3 helix of the GAT domain and those on the so-called Ile44 surface patch of ubiquitin. The binding of the GAT domain to ubiquitin can be enhanced by the presence of a GTP-bound form of ARF. Furthermore, GGA itself is ubiquitinated in a manner dependent on the GAT-ubiquitin interaction. These results delineate the molecular basis for the interaction between ubiquitin and GAT, and suggest that GGA-mediated trafficking might be regulated by the ubiquitin system as endosomal trafficking mediated by other ubiquitin-binding proteins.