PERSPECTIVES: CELL BIOLOGY

GGAs Tie Up the Loose Ends

Sharon A. Tooze

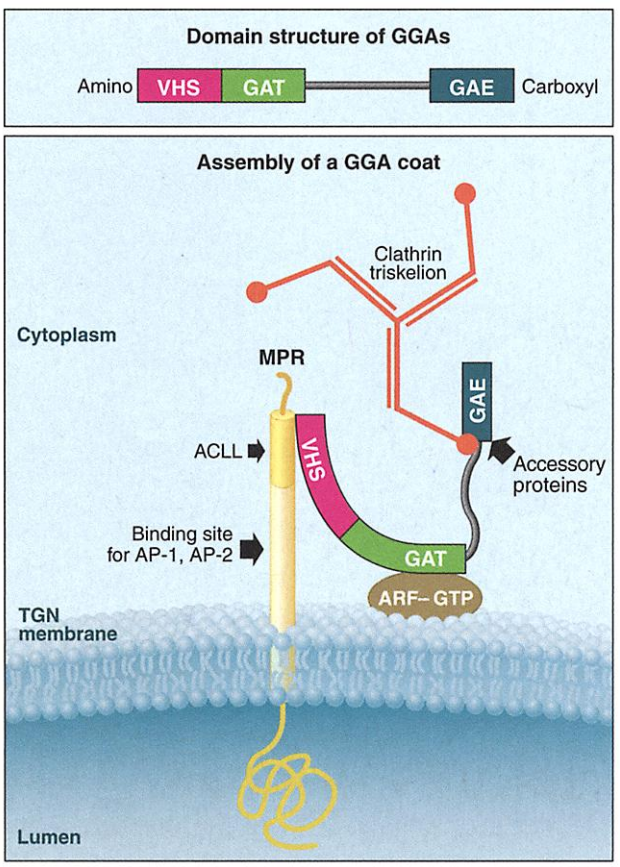
Cargo receptors in the membranes of cellular compartments, such as the endoplasmic reticulum and Golgi apparatus, recruit cargo proteins to regions of these compartments where new vesicles will form. Two related cargo receptors—the cation-independent and the cation-dependent mannose 6-phosphate receptor (CI-MPR and CD-MPR, respectively)—bind to their cargo (lysosomal enzymes) in vesicles budding from the trans-Golgi network (TGN) and deliver their cargo to the late endosome and lysosome compartments. The MPR targeting motifs, which target these cargo receptors to the TGN, have been exhaustively documented, mapped, and mutated. These data suggest that the adaptor protein AP-1 is responsible for sorting CI- and CD-MPRs into TGN vesicles destined for the late endosome. So it comes as a surprise to learn from Puertollano *et al.* (*1*) and Zhu *et al.* (*2*), on pages 1712 and 1716 of this issue, that a recently identified protein family, the GGAs, are responsible for sorting MPRs into vesicles budding from the TGN.

Five members of the GGA family have been isolated so far: GGA1, 2, and 3 in mammalian cells, and Gga1p and Gga2p in yeast. The GGAs (*3*–*7*)—Golgi-localized, gamma-ear–containing, ARF binding proteins—contain three domains, each with its own distinct function (see the figure). The GGA amino terminus contains a VHS domain—a stretch of about 140 amino acids first identified in the proteins VPS27, Hrs, and STAM (*8*). The GAT domain of GGAs binds to ADP-ribosylation factor–guanosine triphosphate (which recruits a variety of adaptor proteins, including AP-1) and stabilizes it in the TGN membrane (*9*). Importantly, the interaction of the GAT domain with ADP-ribosylation factor is required for GGAs to associate with the membranes of cellular compartments. The GGA carboxyl terminus contains the GAE domain, which is linked to the GAT domain by a hinge.

It has been established from yeast mutants with defective GGAs that GGAs are involved in shuttling vesicles between the TGN and the vacuole (*4*), and between the TGN and the late endosome compartment (*10*). In both yeast and mammalian cells, GGAs are required for the formation of clathrin-coated vesicles at the TGN. Clathrin is a protein that provides the structural force necessary to drive vesicle formation (rearrangement of clathrin triskelions on the membrane results in the transformation of the flat clathrin lattice into a spherical clathrin-coated vesicle). In addition to their localization in clathrin-coated regions of the TGN (*4*), GGAs contain clathrin binding motifs in their hinge and GAE domains, suggesting that they may be able to recruit clathrin to newly forming vesicles (*2*, *9*). Overexpression of the VHS and GAT domains of mammalian GGAs interferes with normal GGA activity, resulting in the dissociation of clathrin from the TGN and in the accumulation of CI-MPRs in the TGN membrane (*9*).

Proof that GGAs are indeed involved in recruiting both clathrin and MPRs to TGN vesicles is now supplied by Puertollano *et al.* (*1*) and Zhu *et al.* (*2*). Taking different approaches, the two groups demonstrate that the VHS domain of GGAs directly interacts with the acidic cluster dileucine motif in the cytoplasmic domains of both CI- and CD-MPRs (see the figure). Puertollano and colleagues show with yeast two-hybrid analysis that the VHS domain of all three mammalian GGAs binds specifically to the CI-MPR dileucine motif. Interestingly, this motif does not bind to the VHS domains of other proteins such as Hrs, STAM, and TOM1 (a target of the Myb1 transcription factor). Often preceded by a stretch of acidic amino acids, dileucine motifs are found in a variety of transmembrane proteins. In the two-hybrid assay, however, the GGA-VHS domain only recognized the acidic cluster dileucine motif of MPRs. Thus, there must be subtle features of the MPR dileucine motif that have not been revealed by conventional mutagenesis experiments.

The exact sequence of the MPR dileucine motif (His-Asp-Asp-Ser-Asp-Glu-Asp-Leu-Leu) is also found in the multiligand receptor sortilin. Sortilin, as its name implies, sorts a variety of unrelated cargo into budding vesicles. An independent study has shown that sortilin binds to GGA2 (*11*). In their work, Zhu and colleagues (*2*) found that full-length GGA2 binds to CI-MPRs present in isolated Golgi membranes, but does not interact with



A sting in the tail of GGAs. GGAs are composed of a VHS domain, a GAT domain, and a GAE domain. The GAT domain is linked to the GAE domain by a flexible hinge. Interaction of the GAT domain with ADP-ribosylation factor–guanosine triphosphate (ARF-GTP) results in recruitment of the GGAs to the TGN membrane. Through their VHS domains, GGAs bind to the acidic cluster dileucine motif (ACLL) in the amino terminus of MPRs, which are cargo receptors in the membranes of vesicles budding from the TGN. GGAs on the TGN membrane recruit clathrin triskelions to budding vesicles through clathrin binding motifs in their hinge and GAE domain. The GAE domain also recruits accessory proteins such as gamma-synergin (*5*, *7*) and rabaptin 5 (*2*, *5*). When MPRs are in the TGN membrane, their binding sites for the adaptor protein AP-1 are probably inaccessible, ensuring that GGAs but not AP-1 bind to the MPRs.

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other Golgi-associated proteins even if they contain dileucine motifs. Systematic screening of CI-MPR mutants expressed in CI-MPR–negative fibroblasts revealed that GGA2 binds specifically to the MPR dileucine motif. Importantly, Zhu and coworkers also demonstrated that removal of just the dileucine motif from MPRs was sufficient to abolish binding of GGA2.

The Puertollano group provide additional evidence that the interaction between the GGAs and the MPRs drives the production of TGN-derived transport vesicles. Time-lapse confocal imaging of live cells with GGA1 and CD-MPR proteins tagged with different fluorescent dyes revealed that GGA1 and CD-MPRs are localized in the same vesicles budding from the TGN. There is now a wealth of evidence to support the fact that GGAs are sorting proteins that recruit MPRs into vesicles forming at the TGN.

If this is the case, then what does the adaptor protein AP-1 do? Since the first biochemical demonstration that adaptor proteins sort cargo receptors into vesicles (*12*), and more particularly that AP-1 binds to CI-MPRs, it has been assumed that AP-1 sorts MPRs into TGN vesicles destined for the late endosome. Indeed, there are binding sites for AP-1 in the cytoplasmic domains of both CI- and CD-MPRs (*13*). The Zhu and Puertollano experiments appear to rule out the possibility that AP-1 is involved in the TGN–to–late endosome transport of MPRs, a job that clearly belongs to the GGAs. Rather, it now seems more likely that AP-1 binds to MPRs in the endosomal compartment, helping to recycle these cargo receptors back to the TGN. The GGAs have an exquisite specificity built into their VHS domains, which is necessary to ensure the correct sorting of MPRs into TGN vesicles. Future experiments will no doubt seek to understand the exact molecular nature of this specificity.

References

1. R. Puertollano *et al.*, *Science* **292**, 1712 (2001).
2. Y. Zhu *et al.*, *Science* **292**, 1716 (2001).
3. A. L. Boman, C. Zhang, X. Zhu, R. A. Kahn, *Mol. Biol. Cell* **11**, 1241 (2000).
4. E. C. Dell'Angelica *et al.*, *J. Cell Biol.* **149**, 81 (2000).
5. J. Hirst *et al.*, *J. Cell Biol.* **149**, 67 (2000).
6. A. Poussu, O. Lohi, V.-P. Lehto, *J. Biol. Chem.* **275**, 7176 (2000).
7. H. Takatsu, K. Yoshino, K. Nakayama, *Biochem. Biophys. Res. Commun.* **271**, 719 (2000).
8. O. Lohi, V. P. Lehto, *FEBS Lett.* **440**, 255 (1998).
9. R. Puertollano *et al.*, *Cell* **105**, 93 (2001).
10. M. W. Black, H. R. Pelham, *J. Cell Biol.* **151**, 587 (2000).
11. M. S. Nielsen *et al.*, *EMBO J.* **20**, 2180 (2001).
12. J. N. Glickman, E. Conibear, B. M. F. Pearse, *EMBO J.* **8**, 1041 (1989).
13. J. G. Orsel, P. M. Sincock, J. P. Krise, S. R. Pfeffer, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 9047 (2000).

PERSPECTIVES: MICROBIOLOGY

Cracking *Listeria's* Password

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Listeriosis, a food-borne disease that is particularly severe among infants and the elderly, is caused by the Gram-positive bacterium *Listeria monocytogenes*. Each year in the United States, there are about 2500 cases of listeriosis and 500 deaths (*1*); *Listeria* infection has also been linked to late-term miscarriages in pregnant women. After ingestion of contaminated food, *Listeria* bacteria breach the intestinal barrier, sometimes causing severe gastroenteritis in their human hosts. The bacteria then travel throughout the body, crossing the blood-brain barrier to infect the central nervous system and the placenta of pregnant women to infect the fetus. Although mouse models have been valuable for studying the T cell response to *Listeria* infection (*2*), they cannot reveal how bacteria breach the intestinal barrier because the bacteria are given intravenously rather than orally. This is now set to change with the report by Lecuit *et al.* (*3*) on page 1722 of this issue. These authors have developed an elegant transgenic mouse model and a guinea pig model of listeriosis in which they show that *Listeria* enters gut epithelial cells through binding of its surface protein internalin to an epithelial transmembrane protein called E-cadherin.

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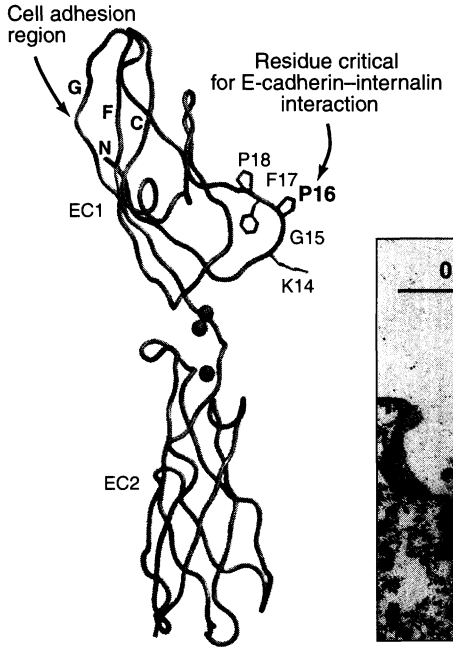
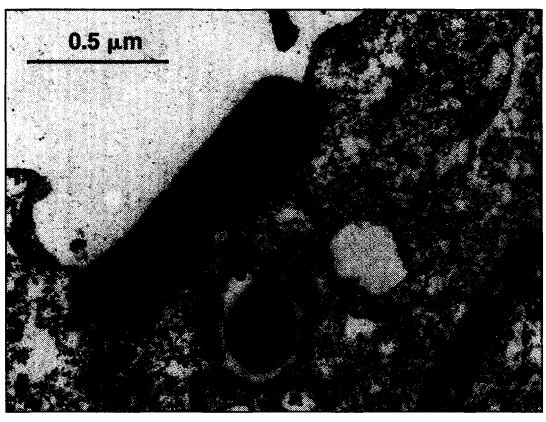
Internalin belongs to the invasin family of bacterial surface proteins, all of which contain leucine-rich repeats. Invasins are used by a variety of intracellular pathogens to invade nonphagocytic host cells such as those of the gut epithelia. The invasin of *Listeria* internalin binds to E-cadherin, which is expressed on the basolateral epithelial cell surface and enables tight junctions to form between epithelial cells (*4*). Noninvasive strains of *Listeria* do not express internalin and cannot invade host epithelial cells, but they become invasive if they are forced to express internalin.

Virulent forms of *Listeria* are specific for certain hosts. Apparently, a single amino acid at position 16 in E-cadherin is responsible for this host specificity (see the figure) (*5*). Human E-cadherin contain a proline residue at position 16, which is critical for binding of internalin, whereas mouse and rat E-cadherin con-





An advantage to being different. (Left) A single amino acid at position 16 in E-cadherin is sufficient to confer host specificity on the bacterial pathogen, *Listeria monocytogenes*. Human and guinea pig gut epithelial cells are susceptible to infection with *Listeria* because the E-cadherin expressed by these cells contains a proline at position 16 to which internalin on the bacterial surface is able to bind. Rat and mouse gut epithelial cells, on the other hand, have a glutamic acid at position 16 and are refractory to infection by all strains of *Listeria* regardless of whether they express internalin. **(Right)** A transmission electron micrograph of *Listeria* invading a cultured human epithelial cell. [Reproduced with permission from (*10*)]