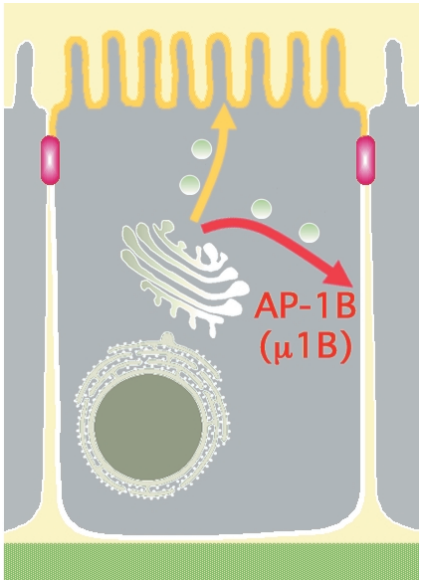
Function of epithelia-specific AP-1B complexes

H. Sugimoto, N. Shioda, T. Mitsunari, F. Nakatsu, M. Kakiuchi, M. Watanabe, N. Nakamura, H. Fölsch, I. Mellman, M. Asano, H. Ohno

The AP complex is a component of coat proteins of the clathrin-coated pit (CCP), which eventually buds off to becomes the clathrin-coated vesicle, the prototype of transport vesicles delivering the cargo proteins in the secretory/endocytic pathways. We cloned a novel APμ chain, μ1B (Ohno et al., FEBS Lett., 1999, 449:215-220). μ1B is exclusively expressed in epithelial cell types, and makes the epithelium-specific AP complex, AP-1B. Biochemical analyses suggested that the other three subunits of AP-1B are shared with the ubiquitously expressed AP-1A. An epithelial cell line, LLC-PK-1, does not express μ1B and mistargets many basolateral proteins to the apical surface. Reconstitution of the μ1B expression in LLC-PK1 selectively restored basolateral targeting (Fölsch et al., 1999, Cell, 99:189-198). Our result, therefore, suggests that μ1B plays a crucial role in polarized sorting of newly synthesized membrane proteins to the basolateral plasma membrane (figure).

We further analyzed the structural basis of basolateral sorting by making and characterizing a mutant μ1B. We have previously shown that the μ subunits of AP complexes directly recognize the tyrosine motifs, one of the most commonly appeared among the sorting signals (Ohno et al., Science, 1995, 269: 1872-1875; Boll et al., EMBO J., 1996, 21: 5789-5795; Ohno et al., J. Biol. Chem., 1996, 271:29009-29015; Ohno et al., J. Biol. Chem., 1998, 273:25915-25921). Tyrosine motifs have the consensus sequence of YXXØ (where Y is tyrosine, X is any amino acids, and Ø is amino acids with a bulky hydrophobic side chain), in which Y and Ø are indispensable for the sorting function *in vivo*. Only the wild-type μ1B, but not the mutant μ1B, supported the basolateral sorting of proteins with the tyrosine motifs, as expected. By contrast, both the wild-type and the mutant μ1Bs were able to target some cargo proteins basolaterally, suggesting that the sorting signal of these proteins interact with a different region of μ1B than tyrosine motifs. We are now characterizing a series of μ1B mutants to test the possibility.



Another interesting feature of μ1B is that it is also required for the monolayer formation of the epithelial cells. The mutant μ1B can substitute for the wild-type μ1B in the monolayer formation, suggesting that the basolateral sorting mediated by the sorting signals other than tyrosine motifs is involved in monolayer formation.